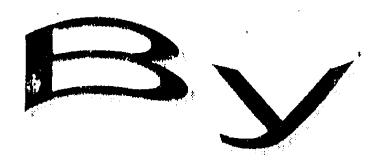
October 6 University  جامعة ٦ أكتوبر

Biochemistry

For Medical Students

Part I



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CONTENTS

Unit I

PHYSIO-CHEMICAL PRINCIPLES

Expression of Concentration:

A solution is formed of a substance dissolved in a liquid. The dissolved substance is called **a solute**. The liquid in which the solute is dissolved is called the **solvent**. Together (solute + solvent) they represent a **solution.**

Concentration:

Concentration of a solute in solution can be expressed in many ways including the percent solutions, molarity, molality and normality.

(a) Percent solutions:

It is equal to the amount of solute per 100 total units of solution.

The expression of percent solution can be done in three ways, namely

- Weight/weight (W/W)
- Volume /volume (V/V)
- Weight /volume (W/V) . This is the most commonly used unit.

Examples of percent solution:

Glucose solution (5%) contains 5 grams of glucose dissolved in 100 ml of distilled water.

Saline (NaCl solution 0.9%) contains 0.9 grams of NaCl salt in 100 ml distilled water.

(b) Molarity (M) :

Molarity is expressed as the number of moles per liter of solution.

One mole of a substance equals its gram molecular weight (the molecular weight expressed in grams).

The systeme International d'Unites (SI) uses mole/litre (mol/L) to express the concentration of a solution.

Millimoles/liter (mmol/L), micromoles/Liter (µmol/L) and nauromoles/Liter (nmol/L) are commonly used units.

N.B. : One liter = 1000 milliliter (ml)

One mole = 1000 millimole (mmol).

One millimole =1000 micromole (µ mol)

One micromole = 1000 nanomole (n mol).

(c) Molality :

Molality represents the amount of solute per one kilogram of the solvent. It is always expressed as moles per kilogram of solvent.

(d) Normality.

Normality is defined as the number of gram equivalent weight per liter of solution. An equivalent weight of a substance is equal to the molecular weight of that substance divided by its **valence**. Normality is no longer used to express concentrations.

N.B.:

-Dilute solution contains very little solute.

-Concentrated solution contains large quantity of solute in solution.

- Saturated solution contains excess of undissolved solute particles.

Law of Mass Action:

This law describes the relation between the velocity of a chemical reaction and the product of concentration of the reacting substances. In a reversible reaction.

$$A + B \underset{\text{Velocity } (V_2)}{\overset{\text{Velocity } (V_1)}{\longleftrightarrow}} C + D$$

V1 is proportional to [A] X [B]

V2 is proportional to [C] X [D]

$\therefore V_1 = K_1 \times [A] \times [B]$

$V2 = K_2 \times [C] \times [D]$

At equilibrium

$V_1 = V_2$

$\therefore K_1 \times [A] \times [B] = K_2 \times [C] \times [D]$

$$\frac{K_1}{K_2} = \frac{[C] \times [D]}{[A] \times [B]} = K \text{ (equilibrium contant).}$$

Applying this law for the dissociation of water, we get the following at equilibrium.

$$H_2O \underset{V_2}{\overset{V_1}{\rightleftharpoons}} H^+ + OH^-$$

$$\frac{V_1}{V_2} = \frac{[H^+] \times [OH^-]}{[H_2O]} = K$$

$K \times [H_2O] = [H^+] \times [OH^-]$.

As the concentration of H_2O is nearly constant due to its weak ionization, its concentration can be considered a unit (1 mole/liter)

$\therefore K \times 1 = [H^+] \times [OH^-]$

The $[H^+]$ at equilibrium is very small and equals 10^{-7} mol/L . Also $[OH^-]$ equals 10^{-7} mol/L.

The pH of water is defined as negative log its hydrogen ion concentration.

$pH = -\log [H^+]$

pH of water = $-\log 10^{-7} = - \times -7 = 7$.

True and titrable acidity:

An acid is defined as a proton (H^+) donor and a base is a proton acceptor.

True acidity is the amount of free hydrogen ion in solution. It determines the pH of the solution. True acidity is low in weak acids and high in strong acids.

Combined acidity represent the non-ionized hydrogen in a substance in solution.

The term Titrable acidity represent all the hydrogen in a solution whether free or combined . All these hydrogen ions can be used during neutralization (Titration) with a base.

pH and its Determination:

The hydrogen ion concentration is often expressed as pH (pH represent the potential or the actual H)

$pH = -\log [H^+]$ or

$$pH = \log \frac{1}{[H^+]}$$

The hydrogen ion concentration is inversely proportional with the pH value.

The pH of pure water is 7 " neutral " because the $[H^+]$ in water is 10^{-7}. Any pH value below 7 is acidic and above 7 is alkaline

Determination of pH:

Methods for determination of pH include:

1. Colorimetric methods.by using indicators. Indicators are weakly ionized acids or bases that change their colour with the change in the pH value. The degree of colour changes is matched with the colour of different standard buffers of known pH.

   Litmus paper change its colour according to the changes in the pH, thus

   Red litmus paper turns blue in alkaline pH , blue litmus paper turns red in acid pH.

2. Electrometric method by using the pH meter. This method depends on the difference in $[H^+]$ between an electrode and the solution whose pH is to be determined, thus creating a potential difference that can be measured .

   Determination of the pH of the blood is important in assessment of the acid-base balance and is essential in diagnosis of acidosis or alkalosis.

Buffers, Acidosis , Alkalosis :

Definition:

Buffers are solutions that keep the pH of a solution constant. Buffers resist any change in the pH after addition of acids or bases to the solution.

Structure:

Buffers are composed of weak acid and its salt or less commonly of weak base and its salt .

Examples of buffers in the blood:

(A) Extracellular buffers:

(1) Carbonic acid/bicarbonate buffer (H_2CO_3) /Na HCO3). It is the main buffers system in the blood . It is responsible for 60% of the buffering capacity of the blood.

(2) Sodium acid phosphate/sodium alkaline phosphate (NaH_2PO_4/Na_2HPO_4).

(3) Plasma proteins (acid proteins/sodium proteinate).

(B) Intracellular buffer " Red cell buffers :

1. Hemoglobin buffer (acid Hb/K Hemglobinate) .
2. Biocarbonate buffer ($HCO_3/KHCO_3$).
3. Phosphate buffer (KH_2PO_4 /K_2HPO_4).

Mechanism of action of buffers :

Buffers help to keep the pH of the blood constant inspite of continuous formation or addition of acids or bases to the blood during metabolism.

Examples of acids added to the blood :

(a) Lactic acid arises from oxidation of carbohydrates in the muscles during muscular contraction and from the red blood cells (RBCs).

(b) Phosphoric acid is formed during metabolism of phospholipids , phosphoproteins and nucleoproteins.

(c) Sulphates from the sulphur containing amino acids.

(d) Keto acids as aceto acetic acid and ß-hydroxybuteric acid which increase in untreated diabetes mellitus . Examples of alkalies that enter the blood include citrates, oxalates, bicarbonates. The main sources of these alkalies are the vegetables, the fruits and the milk.

-Buffers acting in the plasma react directly with the added acids or alkalies as in the following examples :

(a) If a strong acid as HCl is added, it will react with sodium bicarbonate.

$HCl + NaHCO_3$ ---> NaCl (neutral) + H_2CO_3 (weak acid)

(b) If a strong alkali as NaOH is added , it will react with H_2CO_3 as follows:

H_2CO_3 + NaOH ----> $NaHCO_3$ (weak base) + H_2O (neutral), thus the pH of the solution will not be changed significantly.

Hemoglobin as a buffer acts in this way :

At the tissues, CO2 is produced in excess and enters into the red blood cells. Inside the RBC, CO2 forms carbonic acid that dissociates into H^+ and HCO_3^-

$$CO_2 + H_2O \xrightarrow{\text{Carbonic anhydrase}} H_2CO_3$$

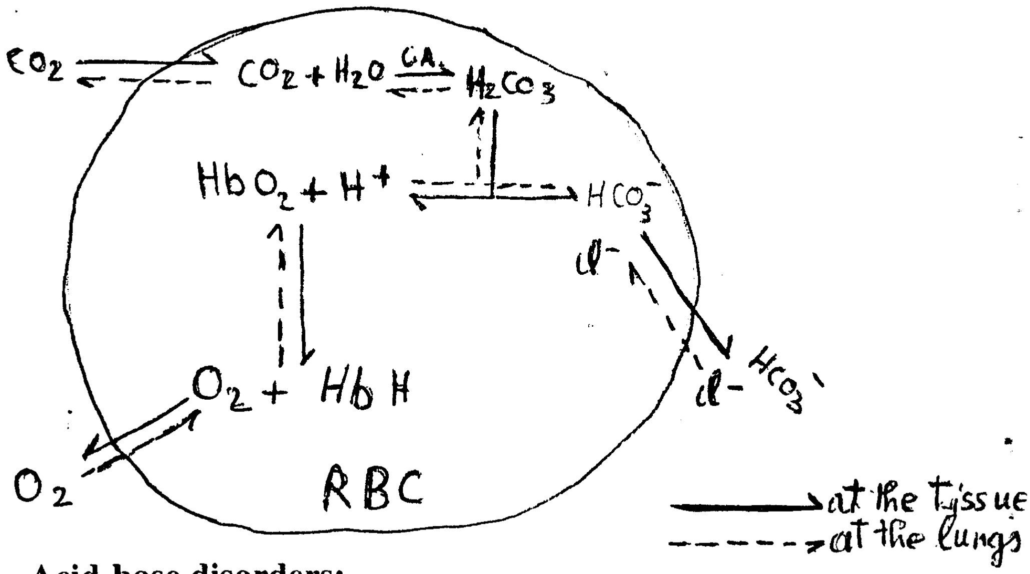
$$H_2CO_3 \longrightarrow H^+ + HCO_3^-$$

The released H^+ is then buffered by hemoglobin

$Hb\text{-}O_2 + H^+$ ----- $Hb.H + O_2$

The bicarbonate passes to the plasma in exchange with the Cl^- "chloride shift"

At the lungs, the reverse will happen. H2CO3- enters the red blood cell in exchange with Cl^-, the H^+ in hemoglobin reacts with HCO_3-to form carbonic acid (H_2CO_3) that changes to H_2O and CO_2 by carbonic anhydrace enzyme . The CO_2 is then released in the expired air



Acid-base disorders:

The pH of the arterial blood is constant at the level of 7.4 ± 0.04 . This is equivalent to [H+] of 40 nmol/L.

pH range 7.36<----- 7.4 ---> 7.44

$[H^+]$ range 44 nmol/L<-- 40nmol/L ---> 36 nmol/L.

Stabilization of the pH at this range is important for the normal metabolic reactions occuring inside the cell. Deviation of the pH from this range will cause serious complications that may lead to coma and death.

A pH below the normal level is referred to as **acidosis**,while a pH above this normal level is called **alkalosis**.

The normal range of the arterial pH is controlled by3 systems. These include.

(a) Buffers. They neutralize the added acids or bases.

(b) Respiratory system. It regulates the amount of carbonic acid.

(c) The kidney system.It regulates the amount of bicarbonates and H^+.

Acidosis

In health, when the kidney and the lungs are normal , the ratio between bicarbonate and carbonic acid is constant at 20:1 and the pH is within normal range.

$$\frac{[\text{Bicarbonate}]}{[\text{Carbonic}]} = \frac{24 \text{ mmol/L}}{1.2 \text{ mmol/L}} = \frac{20}{1}$$

Acidosis is classified according to the cause into:

(A) Metabolic acidosis due to primary decrease in bicarbonate concentration .

(B) Respiratory acidosis due to primary increase in carbonic acid as result of CO_2 retention.

(A) <u>Metabolic Acidosis :</u>

Causes :

The bicarbonate level decreases as a result of the following.

1. Accumulation of non volatile acids that are buffered by bicarbonate leading to decrease in bicarbonate levels as in the following cases.

(a) Uncontrolled diabetes mellitus and severe starvation. In these cases keto acids as acetoacetic acid and ß-hydroxybuteric acid are increased and consume a large amounts of bicarbonate to be buffered.

(b) Adminstration of acidifying salt as calcium chloride or ammonium chloride .

(c) Reduced excretion of acids by the kidney due to renal diseases.

2. Excessive loss of bicarbonate as in diarrhoea.

* ***Metabolic acidosis is compensated by :***

1. Hyper ventilation, which is an increase in the rate and depth of respiration in order to remove more CO_2 that leads to decrease in H_2CO_3, the ratio between bicarbonate and carbonic acid level returns to normal [20:1] and the pH becomes normal too.
2. The kidney retains the bicarbonate and decrease its loss in the urine.

(B) Respiratory acidosis :

It is caused by decreased rate of breathings that leads to accumulation of CO_2 that change into H_2CO_3 .

$$CO_2 + H_2O \overset{C.A}{\longleftrightarrow} H_2CO_3$$

The increase in H2CO3 will change the ratio between bicarbonate and carbonic towards acidosis.

The decrease in the rate of breathing is termed Hypoventilation. The cases that cause hypoventilation include:

1.Obstuctive lung diseases as the bronchial asthma, and bronchopneumonia.

2. Mechanical obstruction of the air ways as in drawning, and suffocation .

3. Heart failure due to decrease in the blood going to the lungs.

4.Drugs as Morphine, barbiturates and alcohol cause inhibition of the respiratory centre in the brain and hypoventilation.

* Respiratory acidosis is compensated by the kidneys. The excretion of H^+ and the reabsorption of HCO_3^- by the kidney is increased to return the ratio between bicarbonate and carbonic acid levels to the normal (20: 1) and the pH becomes normal.

Alkalosis

In alkalosis the level of bicarbonate is increased or the level of carbonic acid is decreased leading to disturbed ratio between bicarbonate and carbonic acid .

$$\frac{[\text{Bicarbonate}]}{[\text{Carbonic}]} = \frac{20}{1}$$

The pH of the blood is increased.

There are two types of alkalosis according to the causes.

1.Metabolic alkalosis due to increase in the level of bicarbonates as in :

(a) Administration of large amounts of bicarbonates as during treatment of peptic ulcers or by infusion.

(b) Loss of excessive amounts of HCl as in vomiting.

*Metabolic alkalosis is compensated by decreasing the respiration " hypoventilation" to retain CO_3 that produce more H_2CO_3 which can return the bicarbonate/carbonic ratio to normal (20:1) and the pH returns to normal level too.

2. Respiratory alkalosis :

* It is caused by increased rate of breathing " hyperventilation" that leads to excessive removal of CO_2 by the lungs, the causes of hyperventilation include :

1. Drugs as salicylates that increase the activity of the respiratory centre .
2. Fever causes hyperventilation in a trial toreduce the body temperature through loss of heat with the expired air.
3. Hysteria.
4. Pulmonary fibrosis.

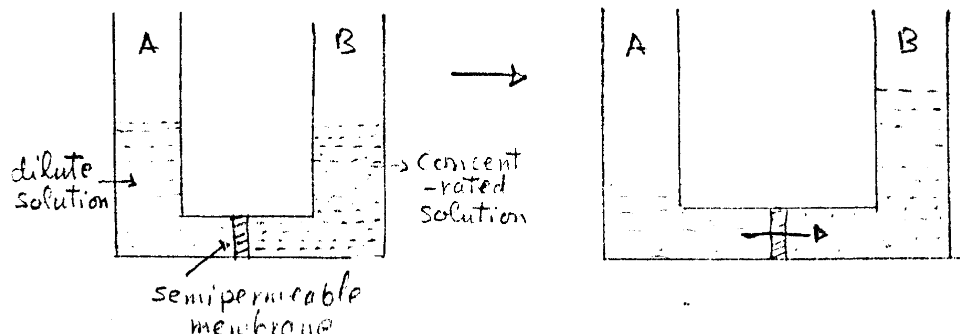
* Respiratory alkalosis is compensated by excretion of the bicarbonate and retension of H^+ by the kidney.

OSMOTIC PRESSURE

* If a semipermeable membrane separates two solutions, the water moves from the dilute solution to the concentrated solution. This movement of water molecule is called osmosis.
* Osmotic pressure is the pressure needed to stop or prevent the osmotic flow.
* Osmotic pressure can be measured by :

1. Osmometer
2. Changing the physical properties of a solution after osmosis as the change in melting point, freezing point.

* Osmosis is demonstrated by the following figure:



water moves to the right until the pressure of the water column in B is equal to the osmotic pressure

* Osmotic pressure is affected by the concentration of the solution and the number of particles in a solution.

Biological significance of osmotic pressure :

The osmotic pressure has many applications in the medical field.

1. The normal distributionof water between the blood and intercellular space. The osmotic pressure of the plasma

proteins help to absorb the water from the intercellular space to the blood capillaries at the venous end. This prevents accumulation of water between the cells " oedema".

2. The normal kidney function depends on filtration of fluids in the glomeruli and reabsorption of fluids in the renal tubules.
3. The movement of water from outside the cell to the inside of the cell depends on the osmotic pressure inside the cells.
4. Fluids that are used for intravenous injection should be isotonic solutions as 5% glucose solution or 0.9% NaCl solution.

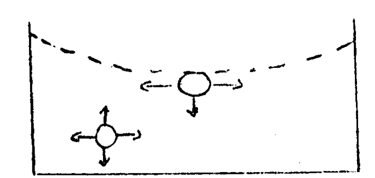
Isotonic solution have the same osmotic power as the plasma. Hemolysis "breaking the RBCs, "occurs in hypotonic solutions having osmotic pressure less than that of the plasma.

Surface Tension:

* Surface tension is the force that helps the surface molecule to be held together.

The molecules of a liquid at the surface are attracted by other molecules inwards and sidwards, while the molecules in the inner part of the liquid are attracted equally in all direction.

This attraction between the molecules help the surface to occupy the least possible area.



*Soups, lecithin " a phospholipid " and bile salts reduce the surface tension of water.

Hypdrotropy :

Hydrotropic substances can make a water insoluble substance as fatty acids and cholesterol more water soluble.

Bile salts and phosphlipids " lecithin" are examples. Bile salt help to make cholesterol in the bile more water soluble to prevent its precipitation that leads to gall stone in the gall bladder.

Adsorption:

Adsorption occurs when a solid particle attract and concentrate on its surface a molecules of gas, liquid, or any dissolved substance.

The particles that attract other molecules are called (adsorbent particles) .

- The adsorbed molecules are called adsorbate.

Adosrbents include :

- Charcoal
- Kaolin.
- Talc powder

Charcoal is used to adsorbe gases as charcoal tablets in flatulence to adsorb gases in the intestine.

Charcoal is used also in the masks used to adsorb toxic gases in wars.

Elution:

It is the process by which the adsorbed substance is removed by a suitable solvent.

Adsorption followed by elution is used as a method to separate and purify substances as in chromatography .

Soltuions:

Solutions are formed of

(a) solutes " dissolved substance"

(b) Solvents " dissolving liquid "

Solutions are classified into three types "

1. True solutions.
2. Colloidal solutions.
3. Suspensions.

The following table shows the comparison between the 3 types of solutions:

True solutions	Colloidal solutions	Suspensions
Example : NaCl sugar solutions	Starch, protein solutions	Blood cells in plasma Chalk powder in water.
Diameter : of Solute particle < 1 nm	1 - 200 nm	> 200 nm
Particles cannot be seen by any microscope	Can be seen by ultramicroscope	Can be seen by ordinary microscope
Particles can pass through filter paper	They can pass	Cannot pass
They can pass through cellophan membrane (dialysable)	They cannot pass	They cannot pass
Stable	Stable	Unstable, sediment.

Properties of colloidal solutions:

Tyndall phenomenon:

If colloidal solution is put in a dark chamber and illuminated from one side only, the colloidal particles appear as bright points. This is becuase the colloidal particles reflects light.

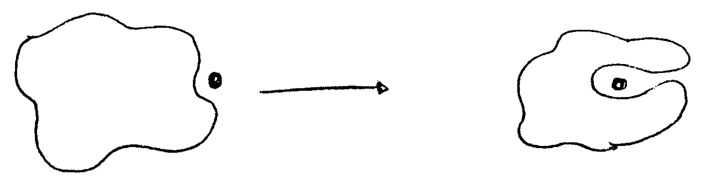
This is the bases of ultra-microscope.

Brownian movements:

When the colloidal solution is examined by ultramicroscope, the solute particles are seen to move in a Zigzag like manner. This is due to bombardment of the solute particles with the solution.

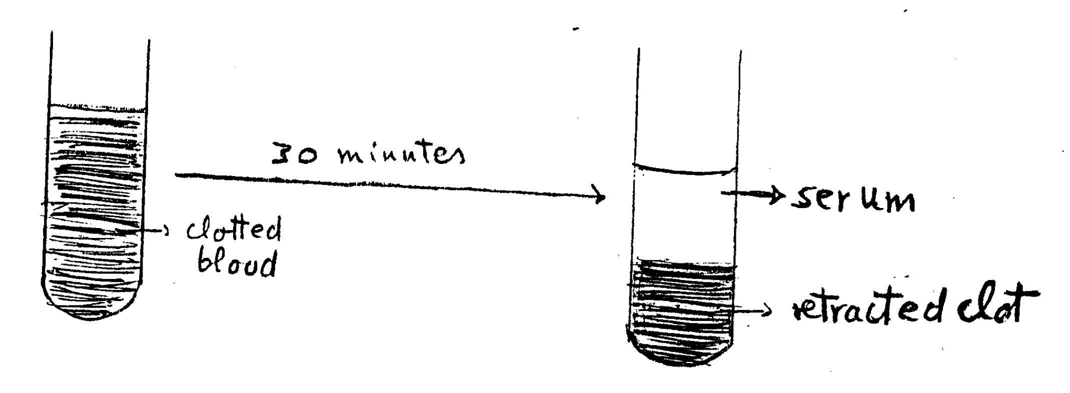
Gel-sol transformation:

The semisolid state of colloids is called gel and it can be changed to sol. or fluid state. The gel-sol transformation occurs during the formation of pseudopo dia.



Imbibition and syneresis :

Certain calloids like gelatin and agar-agar can keep large amounts of water. This is called imbibition. After sometimes they expell this water out, this is called syneresis. Syneresis is observed during contraction of the blood clot expelling the serum as yelloiwsh fluid.



Also synervesis is seen in youghort because the clotted milk contract and expell out a fluid called wh

CARBOHYDRATES

Carbohydrates are polyhydroxy aldehydes or ketones and their derivatives or polymers.

Importance :

1. Glucose is the major fuel of mammals. 45% of the total caloric requirements per day should be obtained from carbohydrates.
2. Carbohydrates enter in the structure of the cell membranes. About 5% of the cell membrane components are carbohydrates.
3. Carbohydrates enter in the structure of important compounds as glycoproteins, glycolipids, DNA, RNA, coenzymes as NAD, FAD, ...
4. Carbohydrates can be changed into triacylglycerol in the the adipose tissue. Excess carbohydrates in the diet is an important cause of obesity.
5. Diseases related to carbohydrates include diabetes mellitus, glycogen storage diseases, galactosaemia, milk intolerance.....

Classification:

Carbohydrates are classified according to the number of saccharide units "simple units" into :

1. Monosaccharides :

The molecule is formed of one sugar units.

2. Oligosaccharides " oligo= few" .

   On hydrolysis, the molecule produce 2-10 monosaccharide units . The most important types are the Disaccharides.

3. polysaccharides . " poly = many "

   The molecule consist of more than 10 sugar units " > 10 monosaccharides".

Monosaccharides :

Monosaccharides are either aldoses which contain an aldehyde group (-C(=O)-H)or ketoses which contain a keto group (>C=0).They are further classified according to the number of carbon atoms in each unit into :

Type	Aldose	Ketose
1- Triose (3 carbons)	Glyceraldehyde C(=O)-H H-C-OH H_2C-OH	Dihydroxy acetone H_2C-OH C=0 H_2C-OH
2- Tetrose (4 carbons)	Erythrose C(=O)-H H-C-OH H-C-OH H_2C-OH	Erythrulose H_2C-OH C=0 H C-OH H_2C-OH

3- Pentoses (6 carbons)	Ribose	C=O, H H-C-OH H-C-OH H-C-OH H_2C-OH	Ribulose	H_2C-OH C=O H C OH H C OH H_2C OH
	Xylose	C=O, H H-C-OH HO-C-H H-C-OH H_2C-OH	Xylulose	H_2C-OH C=O HO C-H H C OH H_2C-OH
4- Hexoses (6 carbons)	Glucose	C=O, H H-C OH HO C-H H C OH H C OH H_2C-OH	Fructose	H_2C-OH C=O HO-C-H H-C-OH H-C-OH H_2C-OH
	Galactose	C=O, H H-C-OH HO-C-H HO-C-H H-C-OH H_2C-OH		
	Mannose	C=O, H HO-C-H HO C-H H C-OH H C-OH H_2C-OH		

Hexoses of physiological importance :

1. **Glucose** : * It is present in grapes and can be obtained by hydrolysis of sucrose, maltose, lactose, starch and glycogen.

*Glucose is the main sugar of the blood. Its normal level in blood is :

70-110 mg/dl " Fasting level "

This level increases in diseases as Diabetes mellitus . The rise of blood glucose level above the normal is termed Hyperglycaemia. On the other hand, Hypoglycaemia is the decrease in the level of blood glucose below 70 mg/dl.

*The appearance of glucose in urine is called Glycosuria.

*Glucose is dextrorotatory , therefore it is sometimes called Dextrose. Dextrose solution (5%) is isotonic solution that can be given intravenously for patients.

2. **Fructose** : It is present in the honey " bee honey" and in ripe fruits. It is mainly obtained by hydrolysis of sucrose "cane sugar". Inulin is a polysaccharide formed of fructose untis.

* Fructose can be changed to glucose in the liver and can be used in the tissues. It is the main sugar of the seminal plasma * It is levorotatory " laevulose ".

3. **Galactose** : It is obtained by hydrolysis of lactose (Milk sugar). It is used by the mammary gland to synthesize

lactose of the milk . It can be changed to glucose in the liver. It is a constituent of glycolipids and glycoproteins.

4. **Mannose** . It is obtained by hydrolysis of plant mannans. It is a constituent of glycoproteins.

Pentoses of physiological importance :

1. ***D-Ribose*** .

It is found in the nucleotides as in RNA, ATP and nucleotide coenzymes as NAD , NADP and COA-SH Ribose is mainly synthesized from glucose through the hexose monophosphate pathway.

2. ***2'-deoxyribose***

It is obtained by removal of (o) from carbon number 2 in the ribose. This process occurs during the synthesis of the deoxyribonucleatides that are used in the formation of DNA (DNA = deoxyribonucleic acid).

A. Physical properties of mononsaccharides:

* All monosaccharides are soluble in water .
* They are mostly sweet . Fructose is very sweet. Mannose is bitter.

- They have one or more asymmetric carbon atom.

An asymmetric carbon atom is attached to 4 different groups or atoms.

The presence of asymmetric carbon atom allows :-

1. Formation of isomers.
2. Optical activity.

Optical activity :

If a plane polarized light passes through a solution of a monosaccharide, it deviates either to the right side (dextrorotatory (+) or d) or to the left side (laevorotatory (-) or l). Glucose is dextrorotatory " dextrose " , while fructose is laevorotatory " laevulose".

Isomers of monosaccharides:

Compounds that have the same formula but differ in the spatial " space " configuration " shape " are called isomers "sterioisomers" .

Types of isomerism:

(l) D and L isomerism.

D-sugar has its (OH) group attached to the carbonatom before the last one to the right side, while L-sugar has this (OH) to the left side.

C=O–H
C-OH
H_2C-OH

D-glyceraldehyde

C=O–H
H-C-OH
HO C-H
H-C-OH
H-C-OH
H_2C-OH

D-glucose

C=O–H
HO C-H
H_2C-OH

L-glyceraldehyde

C=O–H
HO C-H
H C-OH
HO C-H
HO C-H
H_2C-OH

L-glucose

D-Isomers of sugars are the only important forms in animals because the animal cells have enzymes that can deal with D-isomers.

*D and L-isomers are mirror image and are called optical isomers.

2. ***Anomers (α and ß) - Ring structure.***

The carbon atom in the aldehyde or the keto group of sugars is called anomeric carbon. This anomeric carbon may form a ring structure either with C-4 (furan) or with C-5 (pyran).

If the OH group in the ring structure at the anomeric carbon is to the right side , the isomer is α and if this OH is to the left side it is called ß-form.

D-glucose

α- D- glucopyranose

ß - D- glucopyranose

3- ***Epimers***:

Epimers of glucose are isomers that differ in the configuration "shape" of -OH and -H on carbon atoms 2,3 and 4 of glucose.

Galactose is an epimer of glucose since it has its OH group on C-4 at the left side.

Mannose is an epimer of glucose because its OH on C_2 is to the left side.

4. ***Aldose-ketose isomers :***

Glucose is aldose and fructose is a ketose, both are isomers.

B. Chemical properties of monosaccharides :

1. ***Reducing properties:***

All monosaccharides are reducing sugars due to the presence of free aldehyde or keto groups. They can reduce Benedicts reagent .

Cupric hydroxide --------- > cuprous oxide
(blue) (red)

This property is used to detect the sugars in urine in cases of glycosuria.

2. ***Oxidation of monosuccharides:***

Oxidation of sugars gives the corresponding sugar acids.

(A) Oxidation of the carbon 1 gives aldonic acids as gluconic acid from glucose.

(B) Oxidation of the terminal carbon gives uronic acids.

Glucose ---oxid. at C_6---> glucuronic acid

H C OH / H C OH / HO C H / H C OH / H C / COOH
α-D-glucuronic

* Glucuronic acid enter in the structure of glycosaminoglycans.
* glucuronic acid is used to conjugate the water isoluble materials to make them more water soluble and easily excreted.

(C) Oxidation of both C_1 and C6 in Hexoses gives dicarboxylic acids.

glucose ------------> glucaric " saccharic " acid.

galactose ---------- > galactaric " Mucic" acid.

3. ***Reduction of the aldehyde*** or keto groups :

Reduction of the carbonyl carbon gives the corresponding alcohol :

Ribose	glucose or Fructose	mannose	galactose
↓ reduction	↓ red.	↓ red.	↓ red.
H_2C-OH	H_2C-OH	H_2C-OH	H_2C-OH
H C OH	H C OH	HO C H	H C OH
H C OH	HO C H	HO C H	HO C H
H C OH	H C OH	H C OH	HO C H
H_2C-OH	H C OH	H C OH	H C OH
	H_2C-OH	H C OH	H_2C-OH
Ribitol	Sorbitol	mannitol	galactitol (dulcitol)

* Ribitol is a constituent part of ribofavin (Vitain B_2),FMN and FAD "coenzymes"

* Sorbitol production increases in patients with diabetes mellitus " DM" . Its accumulation in the eye tissues " esp. the retina" causes complication in the eye.

*Mannitol is used to reduce oedema especially in the brain due to its osmotic effect.

8 Dulcitol accumulation in the lens of the eye causes cataract.

4. ***Formation of sugar amines*** " Amino sugars" .

* The -OH groups on carbon 2 of hexoses are replaced by amino group ($-NH_2$)

Glucosamine, galactosamine and mannosamine enter in the structure of glycosaminoglycans as hyaluronic acid, heparin and chondroitin sulphate.

```
   C=O H                    C=O H
   |                        |
 H C - NH2            H2N - C - H
   |                        |
HO C - H                 HO C - H
   |                        |
 H C - OH                 H C - OH
   |                        |
 H C - OH                 H C - OH
   |                        |
  H2C - OH                 H2C - OH
```

glucosamine   mannosamine

Disaccharides :

On hydrolysis , disaccharides produce 2 monosaccharide units.

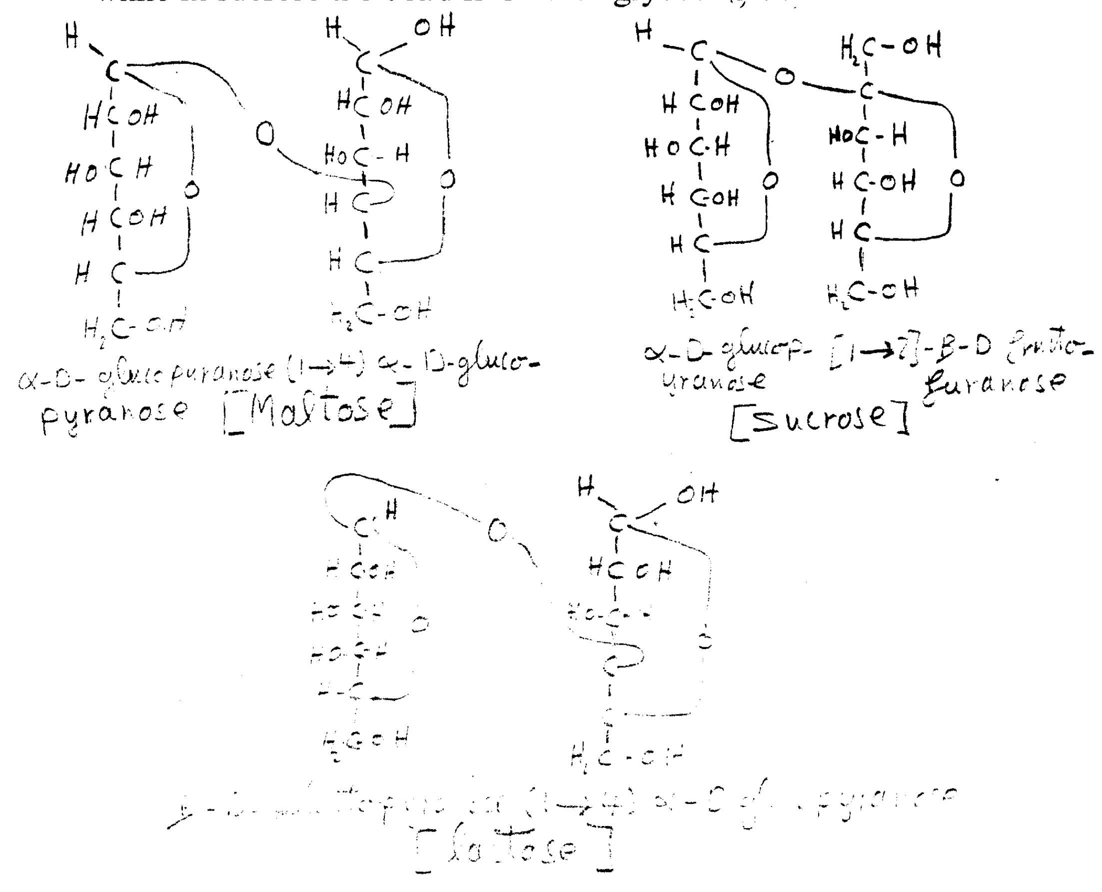
Lactose ----------> glucose + galactose.

Maltose ---------- > glucose + glucose.

Sucrose --------- > glucose + fructose.

The two sugar units are linked together by a glycosidic bond between C-1 in one unit and C4 or C2 in the other unit.

The bond in lactose and maltose is 1 ---> 4 glycosidic bond, while in sucrose the bond is 1---> 2 glycoside bond.



Lactose :

It is the milk sugar. It can occur in the urine of pregnant females.

*It contains galactose and glucose units liked together by ß 1---- > 4 glycosidic bond.

* It is a reducing sugar. It is non-fermentable

Sucrose : (Table sugar)

* It is present in cane, beets , pinapple, sorghum and carrot roots.
* It contains glucose and fructose units linked together by α-1 ---- > 2 glycosidic bond.

-It is non-reducing sugar since, the aldehyde and keto groups are not free.

Maltose :

* It is derived from digestion of starch by amylase enzyme.

It can be obtained from germinating cereals and malt.

* It is formed of 2 glucose units linked together by α-1---4 glycosidic bond.
* It is reducing.

Polysaccharides :

* They produce more than 10 monosaccharide units on hydrolysis .

* They are classified into 2 types :

(A) Homopolysaccharides

They contain one type of sugar units. They include .

1. Starch.
2. Glycogen
3. Dextrins
4. Cellulose.

They are all made up of glucose units " glucosans "

5.Inulin . " It contains only fructose units (fructosan)

(B) Heteropolysaccharides:

They contain more than one type of sugar units or sugar derivatives as sugar amines, glucuronic acid or iduronic acid.

The most important type of heteropolysaccharide is Glycosaminoglycan " mucopolysaccharide" which when linked to a protein in the tissue, the compound is termed Proteoglycans.

(A) Homopolysaccharides

1. Starch:

* It is a store of carbohydrates in plants.

* It is the most important and abundant source of carbohydrates in our food.

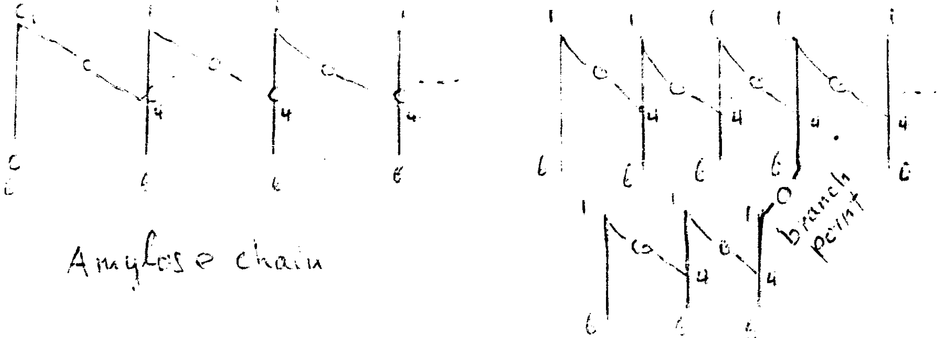
* It is found in cereals, potatoes, sweet potatoes, legums...

* Starch is formed of glucose units linked together by

α-1--->4 glycosidic bonds. Starch granule is formed of 2 parts, an amylose and an amylopectin.

Amylose is non-branching, being formed of glucose units linked by α-1--->4 bonds.

Amylopectin is branching having both α-1--->4 bonds in the chain and α-1--->6 bonds at the branch point.



*Starch is digested into maltose units.

* Starch gives blue colour with iodine.

2. Glycogen :

* It is a storage form of carbohydrates in animal cells.

*It is found in animal cells especially the liver cells and muscles.

*Glycogen is formed of highly branched chain of glucose units linked by α-1--->4 bonds and α-1--->6 bonds at the branch points.

* Muscle glycogen is used up by the muscle to get the energy needed for muscle contraction, while liver glycogen is changed into glucose to the blood during fasting to be used by other tissues.

3. Dextrins:

They are produced during digestion of starch by α-amylase enzyme.

Starch ---α-amylase--->- maltose + dextrins.

4. Cellulose :

* It forms the cell wall of plant cells.
* It is formed of ß-glucose units linked together by ß-1--->4 glycosidic bonds. This bond cannot be digested by α-amylase enzyme in humans . Therefore cellulose cannot be digested in man.
* The cellulose in the intestine forms the bulk of the stool and prevents constipation.

5. Inulin :

* It is formed of fructose units.
* It is found in the roots and tubers of dahlias , artichokes.
*Inulin has been used for testing the kindney function" Inulin clearance."

(B) <u>Heteropolysaccharides</u>

On hydrolysis, they produce more than one type of sugars, and sugar derivatives as :

- Uronic acids.
- Sugar amines.
- Iduronic acid.
- Sialic acid.
- Sulphates.

The types of heteropolysaccharides include :

(1) **Gums** and mucilages.

They are sticky plant exudates. They consists of a mixture of pentoses, hexoses and sugars acids with K, Ca and Mg. They are used as demulcents and emulsifiers and adhesives.

* Plant gums can be sweetened and flavored and used for chewing.

(2) **Pectins** :

They are formed of galacturonic acid and other sugars. They occur in ripe fruits . They can be used as thickening agents and in drugs used in treatment of infantile diarrhoea.

(3) **Glycosaminoglycans** :

They represent the most important types of the heteropolysaccharides. They include :-

Hyaluronic acid :

* It is formed of repeated units of glucuronic acid and N-acetyl glucosamine.

[-Glucuronic acid (1--->3) - N-acetyl glucosamine (1--->4)]

- Hyaluronic acid occurs in the the matrix of the connective tissue, in the synovial fluids of the joints, the vitreous humour of the eye, the umbilical cord and around the mature ovum.

* **Importance of Hyaluronic acid** :

1. It acts as a barrier in the connective tissue against invasion by bacteria.

Some bacteria and the spermatozoa contain Hyaluronidase enzyme that can hydrolyse hyaluronic acid in the subcutaneous tissues that helps the bacteria to spread in the tissues and the spermatozoa to penetrate the ovum during fertilization .

2. Lubrication of joints.

3. It supports the eye tissue and gives the eye its form as it is present in the vitreous humour of the eye.

2. Chondroitin sulphate :

* It is formed of repeated units of glucuronic acid and N-acetylgalactosamine. Sulphate groups are attached to-OH groups of the galactose.
* Chondroitin sulphate occurs in the connective tissue of the cartilage, bones, joints and ligaments.

  (N.B. , Chondro=cartilage).

  * Its function is to absorb the shock of the trauma as in the joints during movements.

3. Heparin :

* It is an anticoagulant produced by the mast cells.
* It contains the following in its structure :
  - acetyl glucosamine (90%)
  - L-iduronic acid (10%).
  - D-glucuronic acid in some forms of heparin .
  - Sulphates.

4. **Heparan sulphate** :

* It is similar to heparin in structure but it contains more glucuronic (90%) and less sulphate and iduronic acid.

*It is produced by endothelial cells and is attached to the cells surface by proteins (Proteoglycan).

5. **Keratan sulphate :**

* It occurs in the corena .

* It contains acetyl glucosamine, galactose and sulphate.

6. **Dermatan sulphate** . It occurs in the connective tissue of the skin.

LIPIDS OF PHYSIOLOGICAL IMPORTANCE

Definition:

The lipids are organic compounds that have the common property of being:-

1. Relatively insoluble in water.

2. Soluble in nonpolar solvents as ether, benzene, petroleum ether, chloroform and acetone. The lipids are related to fatty acids.

Importance of lipids :

1. They are important cellular constituent in the cell membranes. They represent 75% in nerve cell membrane (75% lipids, 20% protein , 5% carbohydrates) and about 45% in other cells.

Lipids in cell membranes are arranged in two layers with their polar groups facing the water on both sides of the membrane (lipid bilayer membrane).

2.Lipids are efficient source of energy. Oxidation of lipids to CO_2 and H_2O produces about 9.1 K cal/g.,while oxidation of protein or carbohydrates produces 4.0 K cal/g for each.

3. Lipids in the adipose tissue under the skin act as thermal insulator to keep the body temperature constant.

4. Lipids in myelin sheath act as electrical insulator "non-polar lipids" to help rapid propagation of nerve impulse.

5. Lipids in adipose tissues around the internal organs support and protect these organs.

6. Lipids in our food contain the fat soluble vitamins (A,D,E,K).

Classification of lipids :

Lipids are classified into:

(1) Simple lipids:

Esters of fatty acids with alcohols .

They include:

(a) Fats " Triacyl glycerol": Esters of fatty acids with glycerol. Oils are liquid fats.

(b) Waxes : Esters of fatty acids with higher alcohol.

(2) Complex " conjugated lipids "

They are formed of lipid part " esters of fatty acids with alcohol" and non-lipid part.

They include:

(a) Phospholipids :

Lipids + phosphoric acid and other constituents.

(b) Glycolipids :

Lipids + carbohydrates.

(c) Lipoproteins:

Lipids + proteins " apoproteins"

(d) Sulpholipids.

(3) Derived lipids and related compounds as fatty acids, glycerol, sterols (as cholesterol) ketone bodies , vitamin A and its precursor " carotenes" , vitamins D,E and K .

Note :

-The Term Neutral Lipids is given for the uncharged lipids as Triacylglycerol, cholesterol and its ester.

- Phospholipids are charged.

Triacylglycerols " Triglycerides" TG

They are esters of the alchol Glycerol with 3 fatty acids.

H_2C-OH
$HC-OH$
H_2C-OH
Glycerol

$R-C(=O)-OH$
Fatty acid

($R-C(=O)-$)
acyl group

$H_2C-O-C(=O)-R$
$R-C(=O)-O-CH$
$H_2C-O-C(=O)-R$

A Triacylglycerol

*Triacylglycerols could be:

1. Mixed TG : They contain different fatty acids . Nearly all the naturally occuring Triacylglycerol " in fats" are mixed (95%).
2. Simple : They contain the same acyl groups " the same fatty acids " in all the 3 ester positions. Their percentage in the naturally occuring fats is very small (5%) .

* Triacylglycerol could be solid at room temperature ---> fat. or liquid at room temperature ----> oils.

Glycerol:

*It is an alcohol containg 3-OH groups (Trihydric alcohol)

* It is soluble in water and ethanol.

* It can be used in preparation of cosmetics.

* It is also used in preparation of nitroglycerine which is used as vasodilator for coronary vessels.

* It loses 2 H_2O giving acrolein that has a pungent irritating odor by dehydration.

H_2C-OH
$HC-OH$
H_2C-OH
Glycerol

$\xrightarrow[2H_2O]{H_2SO_4}$

$C(=O)-H$
CH
$\|$
CH_2
acrolein

Fatty Acids :

* They have the general formula R - COOH
  (R: Carbon chain; COOH: Carboxylic group)

*Most of them have straight chain (R-) with even number of carbons.

* They occur mainly as esters in fats and oils, and few occur as unesterified free fatty acids.

* Few fatty acids are branched and few are cyclic.

* Fatty acids are classified into:

(A) Saturated fatty acids (they contain No double bonds)

(B) Unsaturated fatty acids (they contain one or more double bonds).

A. Saturated Fatty Acids

They are classified according to the number of carbons into 2 types:

I. Short chain fatty acids .

They contain 2-10 carbons. They are liquid at room temperature . They can be dissolved in water .

Examples:

Acetic acid	CH_3COOH	2 Carbons
Buteric acid	$CH_3\ CH_2CH_2COOH$	4 carbons
Caproic	$CH_3(CH_2)_4\ COOH$	6 carbons
Caprylic	$CH_3(CH_2)$ 6 COOH	8 carbons
Capric	CH3(CH_2)8 COOH	10 carbon

2. Long chain fatty acids.

They contain more than 10 carbons. They are solid at room temp. They are not soluble in water, but soluble in fat solvents.

Examples:

Palmitic	$CH_3(CH_2)_{14}$ COOH	16 carbons
Stearic	CH3 $(CH_2)_{16}$ COOH	18 carbons

B. Unsaturated Fatty Acids

They are classified according to the number of the double bonds into :

1. Monounsaturated " Monoenoic" fatty acids. They contain only one double bond as palmitoleic and oleic acids.

2. Polyunsaturated " polyenoic" fatty acids.

They contain two or more double bonds.

The following table shows the number of carbons, the number of double bonds and the position of the double bonds in the carbon chain according to the Δ numbering system.

Name of the fatty acid	Number of carbons	Number of double bonds	Position of double bonds
1. Monounsaturated			
Palmitoleic	16 C	1	9
Oleic	18 C	1	9
2.Polyunsaturated			
Linoleic	18 C	2	9,12
Linolenic	18 C	3	9,12,15
Arachi donic	20 C	4	5,8,11,14
Clupanodonic	22 C	5	7,10,13,16,19

Essential fatty acids

*They are polyunsaturated fatty acids.

* They are very essential for normal growth and good health.

* The essential fatty acids cannot be synthesized in the body and must be supplied in the food. Their deficiency in food causes diseases.

* They protect the body against atherosclerosis because they form esters with cholesterol whih can be easily metabolised and removed from the tissues.

* The polyunsaturated fatty acids are present in large amounts in the vegetable oils as corn oil, olive oil and sunflower oil.

* Arachidonic acid contains 20 carbon (eicosa) and is the origin of substances called eicosanoids. The most important eicosanoid is a substance called prostaglandin. Prostaglandins were first discovered in the seminal vesicle and were throught to be formed only in the prostate. They are actually formed in every tissue and they act as local hormones. The functions of prostaglandins are many, but the most important is the initiation of the inflammatory reactions. The enzymes needed for their synthesis are phospholipase A2 and cyclooxygenase. These enzymes are inhibited by cortisone and aspirin which are good anti-inflammatory drugs.

Conjugatend lipids

(A) Phospholipids :

They are lipids that contain phosphoric acid. The phospholipis include the following types :

1. Phosphatidic acid.

* Phosphatidic acid is formed of glycerol, two fatty acids and phosphoric acid.

*It is intermediate compound in the synthesis of triacylglycerol and phospholipids.

2. **Lecithin** " Phosphatidyl choline".

* Lecithin is formed of glycerol, 2 fatty acids, phosphoric acid and choline.

* It is present in the cell membranes , the plasma, the brain and the liver. Egg Yolk contains lecithin.

The importance of Lecithin :

1. Lecithin is the most abundant phospholipid of the cell membrane.

2. It represents a good store of choline which acts as source of methyl groups.

$HO\text{-}CH_2\text{-}CH_2\text{-}\underset{|}{\overset{}{N}}(CH_3)3$ " choline"
OH

3. Dipalmityl lecithin reduces adhesions between the tissues especially in the lungs. Lecithin acts as surfactant in the lungs. Defeciency of dipalmityl lecithin in premature babies causes respiratory distress syndrome. The lung surfaces adhere together and the breathing becomes difficult.

- Lecithinase enzyme which is present in the venoms of snakes and bees can hydrolyse the lecithin of the plasma and remove the fatty acid in position 2 to form lysolecithin.

Lecithin ---Lecithinase---> lysolecithin + fatty acids.

The lysolecithin is a hemolytic agent. It causes lysis of the RBC,s.

3. Cephalin " phosphatidyl ethanolamine "

* It is present in brain tissue and blood plasma.
* It contains glycerol, 2 fatty acids, phosphoric acid and ethanolamine ($OH-CH_2-CH_2-NH_2$).
* Cephalin like phospholipids contains inositol or serine instead of ethanolamine to form phosphatidyl inositol or phosphatidyl serine respectively.

4. **Cardiolipin** :

Cardiolipin is found in the mitochondria. It contains 2 phosphatidic acids linked together by glycerol " Diphosphatidyl glycerol" cardiolipin is antigenic lipid.

5. Plasmalogen :

*Plasmalogens represent about 10% of the phospholipis of the brain and muscles.

* They contain glycerol, unsaturated alcohol linked to Cl of glycerol by ether linkage, one fatty acid, phosphoric acid and ethanol amine or serine or inositol.

6. Sphingomyelins :

* Sphingomyelins are found in large amounts in the brain and nerve tissue.

*Sphingomyelins contain the alcohol sphingosine, one fatty acid, phosphoric acid and choline. Acombination of sphingosine and a fatty acid is called ceramide.

```
1  CH2 - O - C(=O) - R1
   |
2  CH  - O - C(=O) - R2
   |
3  CH2 - O - P(=O) - OH
             |
             OH
```

phosphatidic acid.

N.B. (P) = phosphoric acid

```
CH2 - FA1
|
CH  - FA2
|
CH2 - (P) - Choline
```

Lecithin

FA = Fatty acid

```
CH2 - FA1
|
CH  - FA2
|
CH2 - (P) - ethanolamine
```

Cephalin « phosphatidyl-ethanolamine »

```
CH2 - FA1
|
CH  - FA2
|
CH2 - (P) - serine
```

Phosphatidyl serine

```
CH2 - FA1
|
CH  - FA2
|
CH2 - (P) - inositol
```

phosphatidyl inositol « Lipositol »

```
CH2 - FA1                                  CH2 - FA1
|                                          |
CH  - FA2                                  CH  - FA2
|                                          |
CH2 - (P) - glycerol - (P) - CH2
```

Cardiolipin

```
CH2 - OH
|
CH  - NH2
|
CH2 - OH
|
R
```

Sphingosine alcohol

```
CH2 - (P) - Choline
|
CH  - NH - FA
|
CH2 - OH
|
R
```

Sphingomyelin

```
CH2 - O - galactose
|
CH  - NH - Fatty acid
|
CH  - OH
|
R
```

cerebroside (galactolipid)

```
CH2 - O - oligosaccharide
|
CH  - NH - Fatty acid
|
CH  - OH
|
R
```

Ganglioside

*Niemann-Picks disease is caused by hereditary deficiency of sphingomyelinase enzymes that breaks down sphingomyelins. Accumulation of sphingomyelius in the brain causes mental retardation.

B. **Glycolipids** :

They are compound lipids that contain carbohydrates. They consist of sphingosine alcohol attached to one fatty acid "ceramide" and one or more sugar units.

Glycolipids include 2 types :

(A) Cerebrosides

(B) Gangliosides.

The fatty acid present in glycolipid are all C_{24} fatty acids especially in the brain. C_{24} faty acids include:

1. Lignoceric 2. Cerebronic

3. Nervonic

The following table shows the characteristics of cerebrosides and gangliosides.

Cerebrosides	Gangliosides
Composition: Sphingosine + one fatty acid + one galactose or glucose	Sphingosine + one fatty acid + sialic acid + oligosaccharide containing one glucose and 2 galactose units and sometimes, neuraminic acid.

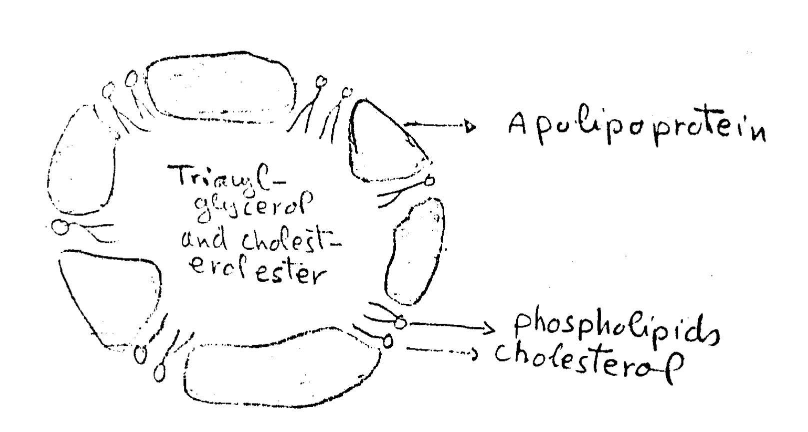
* They occur in the myelin sheath, brain and plasma membrane	* They occur in the nervous tissue and as receptors in the cell membrane . * Some antigens as ABO blood group substances are formed of the oligosaccharides present in the glycosphingolipids.

C. **Lipoproteins:**

* Lipoproteins are compound lipids formed of lipids and proteins . Lipoproteins are present in the cell membrane, the mitochondria and in the blood plasma.

Plasma lipoprotein

Plasma lipoproteins are composed of a protein layer called apoprotein that surround the lipids in the core



Composition of lipoprotein particle

*The non-polar lipids are located within the core, while the more polar lipids as the phospholipids and the free cholesterol are near the surface with the apoproteins.

*Plasma lipoproteins are important in the transport of the water insoluble lipids from one organ to the other.

Plasma lipoproteins are classified according to their densities into the following types :

1. Chylomicrons (density < 0.95 g/ml).
2. Very low density lipoprotein- VLDL (density 0.95-1.006 g/ml).
3. Low density lipoprotein -LDL (density 1.01-1.063 g/ml).
4. High density lipoprotein - HDL (density 1.064-1.21).
5. Free fatty acids + albumin (higher density)

lipoproteins can be also classified according to their electrophoretic mobility into chylomicrons, pre-ß-lipoprotein (VLDL) , ß-lipoprotein (LDL) and α-lipoprotein (HDL). The electrophoretic mobility depends on the fact that the protein parts of the lipoprotein carry a negative charge at alkaline pH. In an electric field the different lipoproteins move towards the anode (+ve pole) at different speeds depending on their size and densities. The chylomicrons stay at the origin and do not move, the low density lipoproteins (LDL) stops at the area of ß-globulins and hence the name ß-lipoproteins, VLDL stops at the area just before the ß-globulins (pre-ß-lipoproteins) while the HDL stops at the area of the α-globulin (α -lipoproteins).

The following table shows some properties and the functions of lipoproteins.

Lipoprotein	density g/ml	mean diameter (nm)	Source	The main function
1. Chylomicron	<0.095	500	Intestine	Absorption of digested lipids.
2. VLDL (Pre-ß)	0.94-1.006	40	Liver	Transport of endogenous triglycerides.
3. LDL (ß)	1.01-1.062	20	Catabolism of VLDL	Transport of cholesterol from the liver to the tissues.
4. HDL (α)	1.962-1.2	9	Liver	Carriers cholesterol from tissues to the liver to be excreted.

*The lipids carried by lipoproteins include triglycerides, cholesterol and its esters and phospholipids. The concentrations of each type varies in the different types of lipoproteins.

*The apolipoproteins that form the lipoproteins includes 5 main types, namely A,B,C,D and E .

The following table shows the percentage of each lipid and apolipoproteins in each type.

Lipoprotein	Apoprotein, Type & %	Triglycerides	Cholesterol	Phospholipid
Chylomicron	ß-48, C,E 1%	90%	5%	4%
VLDL	ß-100, C,E 10%	65%	15%	10%
LDL	ß-100 20%	10%	48%	22%
HDL	A,C,E 50%	4%	22%	24%

Derived lipids

Derived lipids are derivatives of simple or compound lipids after hydrolysis and which are not soluble in water. They include the following :

1. Fat soluble vitamins A,D,E,K.
2. Carotenes , α, ß- and ". They are provitamin A.
3. Long chain fatty acids.
4. Squalene. It is present in some plant oils.It is also an intermediate substance for the synthesis of cholesterol.
5. Sterols and steroids.

Steroids

Steroids are all the compounds that contain a steroid nucleus "ring". The steroid ring is a cyclopentanoperhydrophenathrene ring.

Steroid compounds include :

1. Sterols (animal and plant sterols).
2. Bile salts.
3. Steroid hormones.
4. Vitamin D "D2 and D3"
5. Cardiac glycosides as digitalis.

I. **Sterols** :

According to their origins , sterols include :

(A) Animal sterol " cholesterol.

(B) Mycosterols in yeast, algae and fungi as ergosterol.

(C) Phytosterols in higher plants .

Cholesterol:

Cholesterol is present in every animal cell as part of the cell membranes. It is also present in the blood plasma carried by the lipoproteins as LDL, HDL and VLDL.

Sources of cholesterol :

1. Endogenous : synthesis of cholesterol occurs mainly in the liver form acetyl COA.
2. Exogenous : Foods that are rich in cholesterol are arranged as follows in a descending order.

Brain, egg yolk, liver, organ meats, butter, animal fats. Note that plant oils are free from cholesterol.

The normal total level of blood cholesterol is 150-200 mg/dl, about two thirds of this level are esterified with fatty acids and the rest are free cholesterol. The cholesterol in the blood is carried in the different lipoproteins. The desirable level of LDL-cholesterol should be less than (<) 130 mg/dl, and the level of HDL-cholesterol should be more than 40 mg/dl. High level of LDL-cholesterol predisposes to Atherosclerosis and thrombosis, while high level of HDL-cholesterol is a good sign which means that the excretion of cholesterol is very effecient.

Excretion of cholesterol :

About 1 g of cholesterol is excreted from the body per day . About 50% of this 1 g is changed in the liver to bile acids which are excreted as bile salts in the faeces. The rest

is excreted as free cholesterol that is changed mainly to coprosterol by bacteria in the lower intestine.

The primary bile acids are synthesized in the liver from cholesterol by oxidation . The primary bile acids are cholic and chenodeoxycholic acid. The bile acids are conjugated with glycine or taurine to form glycocholic or taurocholic acids and glycochenodeoxy cholic or taurochenodeoxy cholic acids . The bile acids in the alkaline bile are actually present as bile salts. Part of the bile salts in the intestine is reabsorbed to reach the liver that excretes them again into the bile to reach the intestine " enterohepatic circulation ".

Some primary bile acids are changed by bacterial enzymes in the intestine into secondary bile acids as deoxycholic acid from cholic acid by dehydroxylation and as Lithocholic acid from chendeoxycholic acid. Bile salts help digestion of lipids.

Biochemical importance of cholesterol :

1. Cholesterol is a structural element in the cell membranes. It helps in keeping the fluidity of cell membranes under different temperatures.

2. Cholesterol is the precursor of :

(a) cholcalciferal " vitamin D3".

(b) bile salts.

(c) Steroid hormones which include female sex hormones (estrogens and progesterone) male sex hormones (testostrone), corticosteroids (mineralocorticoids and glucocorticoids).

3. Hypercholesterolaemia predisposes to atherosclerosis.
4. Cholesterol stones can be formed in the gall bladder when cholesterol excretion in bile is increased.

Important properties of fatty acis and fats.

l. Solubility and the physical state :

Short chain fatty acids are soluble in water, while long chain fatty acids and fats are soluble only in fat solvents.

The melting point of the fatty acids depends on the length of the carbon chain and on the presence of double bonds " unsaturation". Short chain fatty acids and long unsaturated fatty acids have low melting point " below the room temperature " and are liquid at rootm temperature . Long chain saturated fatty acids have high melting point " higher than room temperarature" and are, therefore, solid at room temperature . Plant oils are liquid at room temperature because of their high content of unsaturated fatty acids, while fats are solid at room temperature because of their high content of saturated fatty acids.

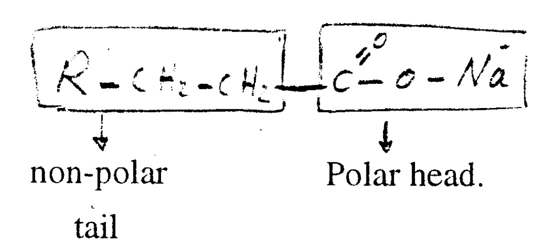
2. Hydrogenation:

Addition of hydrogen to the unsaturated fatty acids in plant oils change these oils into solid fat " artificial butter or margarine ". This change in the physical state of the oil is

due to the increase in the melting point of their content of fatty acids which become saturated fatty acids .

3. Soap formation

Soaps are salts of long chain fatty acids with alkalies. Sodium and potassium salts of fatty acids are the ordinary soaps. Soaps have a polar head and a non-polar tail.



non-polar tail → Polar head.

The non polar tail can be dissolved in the fats or grease while the polar head can be dissolved in water and thus soaps can remove the fats or the greese with water.

4. **Rancidity** :

Rancidity is the change in the taste, the odor and the color of fats. The rancid fats have unpleasant taste and odor.

Causes of rancidity :

1. Exposure of the fats to atmospheric oxygen .
2. Exposure to water " humidity" and to increase in the temperature.
3. Contamination with bacteria.

Types of rancidity :

1. Hydrolytic rancidity.

Bacterial lipases hydrolyse fats into free fatty acids and glycerol.

Triacyl glycerol"fat" $\xrightarrow[H_2O]{\text{bacterial lipase}}$ 3 fatty acids + glycerol.

The free fatty acids cause the sour taste of rancid fats.

2. Oxidative rancidity :

The unsaturated fatty acids are oxidized to form peroxides. Free radicls are produced during peroxide formation. The free radicls can cause damage to the tissues, atherosclerosis . cancer, aging,.... when the peroxidation occurs in the tissue lipids.

3. Ketonic rancidity :

Aldehydes, ketones and alcohols are produced.

Prevention of rancidity :

1. Keep the fats and oils away from moisture, aire and bacteria.
2. Add anti oxidant to the lipids . The autioxidants include :
   - (a) Vitamin E.
   - (b) Vitamic C
   - (c) Uric acid
   - (d) Phenols
   - (e) ß-carotene " provitamin A".

These substances inhibit the oxidation of the unsaturated fatty acids.

PROTEINS

Proteins are organic compounds of high molecular weight. Protein molecule is made up of several units of amino acids that contain nitrogen. The nitrogen content of proteins is 16%.

Importance of proteins :

1. Proteins are important structural elements in the cell. They form about 20-50% of the cell membrane components.

2. Catalytic function :

All enzymes are protein in nature. One small exception is the ribozyme which is a small piece of RNA that catalise its breakdown.

3. Transport functions :

Hemoglobin transports O_2, transferrin transports iron, albumin transports bilirubin and fatty acids,....

4. Movement :

The contractile proteins, actin and myosin are responsible for muscle contraction and movements.

5. Defensive function:

The γ-globulins forms the antibodies against invading bacteria and viruses.

6. The osmotic pressure of plasma proteins control the distribution of water between the intravascular and interstitial spaces.

7. Vision:

The visual pigments rhodopsin and scotopsin contain the protein opsin with vitamin A aldehyde.

8. Some hormones are protein in nature e.g. insulin, growth hormone,....

AMINO ACIDS

The amino acids that form the proteins are all L - α amino acids .

$$H_2N - \underset{\displaystyle R}{\overset{\displaystyle COOH}{\underset{|}{\overset{|}{C}}}} - H$$

The amino acids found in proteins are classified into 2 types :

(A) 20-coded amino acid (19 L-α-amino acids and one L-imino acid).

(B) Modified amino acids that are derived from one or more of the 20-coded amino acids.

(A) The 20 coded -amino acids

They include the following groups :

I. Aliphatic monocarboxylic acids

*Glycin "Gly". $\underset{\displaystyle NH_2}{\underset{|}{CH_2}} - COOH$

*Alanine " Ala " $CH_3\ \underset{\displaystyle NH_2}{\underset{|}{CH}} - COOH$

*Valine " Val : $CH_3 - \underset{CH_3}{CH} - \underset{NH_2}{CH} - COOH$

* Leucine " Leu" $CH_3-\underset{CH_3}{CH}-CH_2-\underset{NH_2}{CH}-COOH$

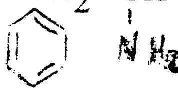
*Isoleucine " Ile" $CH_3-CH_2 - \underset{CH_3}{CH} - \underset{NH_2}{CH} - COOH$

* Serine"Ser" $\underset{OH}{CH_2}-\underset{NH_2}{CH} - COOH$

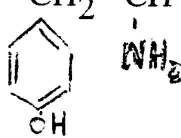
* Threonine " Thr" $CH_3\ \underset{OH}{CH} - \underset{NH_2}{CH} - COOH$

II. Aromatic amino acids

* Phenylalanine " phe" $CH_2 - \underset{NH_2}{CH} - COOH$



*Tyrosine " Tyr" $CH_2 - \underset{NH_2}{CH} - COOH$



*Tryptophan "Trp" $CH_2 - \underset{NH_2}{CH} - COOH$

N

III. Acidic amino acids and their amides.

* Aspartic acid " Asp" $H_2N\text{-}CH_2 - COOH$

CH_2 COOH

* Asparagine " aspartic acid amide " " Asn"

$H_2N - CH - COOH$

|

$CH_2 - C(=O) - NH_2$

* Glutamic acid "Glu"

$H_2N - CH - COOH$

|

CH_2

|

$CH_2 - COOH$

* Glutamine " Glutamic acid amide " " Gln"

$H_2N - CH - COOH$

|

CH_2

|

$CH_2 - C(=O) - NH_2$

IV. Basic amino acids

*Lysine " Lys"

$H_2N\text{-} CH_2 - CH_2 - CH_2\text{-} CH_2\ CH - COOH$

|

NH_2

*Arginine " Arg"

$H_2N - C(=NH) \text{-}NH - CH_2\ CH_2\text{-}\ CH_2\text{-}CH - COOH$

|

NH_2

*Histidine "His"

$$\text{Imidazole-}CH_2\text{-}CH(NH_2)\text{-}COOH$$

V. Sulphur -containing amino acids.

*Cysteine "cys"

$$HS - CH_2 - CH(NH_2) - COOH$$

*Methionine "Met"

$$CH_3 - S - CH_2 - CH_2 - CH(NH_2) - COOH$$

VI. Imino acid

*Proline "Pro"

$$\text{Pyrrolidine (NH)-}COOH$$

(B) Modified amino acids

They are derived from some of the 20-coded amino acids through modifications in their structue within the protein.

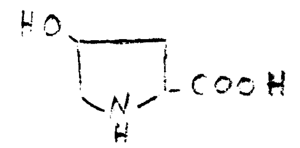
I. Hydroxy lysine

$$H_2N - CH_2 - CH(OH) - CH_2 - CH_2 - CH(NH_2) - COOH$$

*Cystine

$$
\begin{array}{l}
S - CH_2 - \underset{\displaystyle NH_2}{\underset{|}{CH}} - COOH \\
| \\
S - CH_2 - \underset{\displaystyle NH_2}{\underset{|}{CH}} - COOH
\end{array}
$$

* Hydroxy proline



Note : The above listed amino acids occur in the structure of proteins. Some other amino acids exist separate in the body and have important roles in metabolism.

These amino acids include :-

* Homoserine

$$\underset{\displaystyle OH}{\underset{|}{CH_2}} - CH_2 - \underset{\displaystyle NH2}{\underset{|}{CH}} - COOH$$

* Homocysteine

$$\underset{\displaystyle SH}{\underset{|}{CH_2}} - CH_2 - \underset{\displaystyle NH_2}{\underset{|}{CH}} - COOH$$

*Citrulline

$$H_2N - \underset{\displaystyle O}{\underset{||}{C}} - HN - CH_2 - CH_2 - CH_2 - \underset{\displaystyle NH_2}{\underset{|}{CH}} - COOH$$

Ornithine

$$H_2N - CH_2 - CH_2 - CH_2 - \underset{\displaystyle NH_2}{\underset{|}{CH}} - COOH$$

*Argininosuccinic acid

$$HN = C - NH - CH_2 - CH_2 - CH_2 - CH(NH_2) - COOH$$
$$\quad | \; NH - CH(COOH) - CH_2 - COOH$$

*Dihydroxyphenylalanine " DOPA"

$$(HO)_2C_6H_3 - CH_2 - CH(NH_2) - COOH$$

Some other amino acids have their amino group attached to a non - α-carbon. They play important roles in metabolism.

- ß-alanine is an example. It exists in the structure of pantothenic acid, coenzyme A, anserine and carnosine.

$$H_2N - \overset{\beta}{C}H_2 - \overset{\alpha}{C}H_2 - COOH$$ ß-alanine

- γ-Aminobuteric " GABA" have its amino group attached to the γ-carbon. It is an inhibitory neurotransmittor in the brain

$$H_2N - \overset{\gamma}{C}H - \overset{\beta}{C}H_2 - \overset{\alpha}{C}H_2 - COOH$$ GABA

Nutritional classification of amino acids :

Amino acids are classified into 2 groups according to their nutritional importance .

(A) Essential " indespensible"

Valine, Leucine, isoleucine, threonine
Phenylalanine , Tryptophan
Lysine, Arginine, Histidine
Methionine.

These essential amino acids cannot be synthesized inside the body. They must be supplied in the food. Their defeciency in food causes diseases as pellagra which is caused by defeciency of Tryptophan and fatty liver that is caused by defeciency of methionine . Histidine and arginine are essential only for growing children while adults can synthesize them in insufficient amounts " semiessential".

(B) Non essential or dispensible amino acids. They include all other amino acids. They can be formed in the body in sufficient amounts.

Metabolic classification of amino acids :

The amino group of amino acid is removed and excreted as urea. The carbon skeleton of the amino acid may change into ketone " ketogenic amino acid", glucose " glucogenic" or into ketone and glucose " mixed".

Kketogenic amino acid	Mixed "glucogenic and ketogenic amino acids"	Glucogenic amino acids
Leucine	Phenyl alanine, tryosine, tryptophan, isoleucine and lysine	Ala, Arg, Asp, Cyst, Glu, Gly, Hist, Met , Pro, Ser, Thr, Val, Hypo

*The side chain " R- group " of the amino acid could be polar " hydrophilic " or non-polar " hydrophobic". The non-polar groups of the amino acids get away from water and thus affecting the shape of the protein molecule that contain these amino acid.

Non-polar " hydrophobic " amino acids	Polar " Hydrophilic " amino acids
Alanine, Leucine, isoleucine, Valine, phenylalanine, Tryosine, Tryptophan, proline	Histidine, lysine, Arginine, glycine, serine, threonine , Aspartic, Asparagine, Glutamic. glutamine.

Properties of amino acids:

1. Solubility :

Amino acids are soluble in polar solvents " water and ethanol". Cystine is the least soluble.

2. Optical properteis .

All the amino acids except glycine contain asymmetric carbon atom and are therefore optically active. The natural amino acids have L-configuration. D-amino acid exist only in bacteria and in some antibiotics.

COOH
|
H_2N - C - H
|
R

L-amino acid

COOH
|
H - C - NH_2
|
R

D-amino acid

3. Ninhydrin reaction.

All amino acids react with ninhydrin giving blue color. Proline and hydroxy proline give yellow color. This reaction is used for detection and quantitation of amino acids.

4. Amphoteric properties :

Amino acids possess both an acidic group (-COOH) and a basic group (-NH2) . In acid medium, amino acids carry positive charge , while in alkaline medium they carry negative charge. AT a certain pH, amino acids carry both negative and positive charges equally this pH is called isoelectric point (I.E.P). The amino acid at the isoelectric point is called Zwitterion or Dipolar ion. Zwitterion is electrically neutral and does not migrate in an electric field .

$$\underset{\displaystyle R}{H_3\overset{+}{N}-CH-COOH} \xleftarrow[\text{pH}]{\text{acid}} \underset{\displaystyle R}{H_3\overset{+}{N}-CH-COO^-} \xrightarrow[\text{pH}]{\text{alkaline}} \underset{\displaystyle R}{H_2N-CH-COO^-}$$

REPTIDES

Amino acids bind together to form peptides. The binding occurs between a carboxylic group of one amino acid and the amino, group of the next to form a peptide bond.

$$\underset{\displaystyle R_1}{H_2N-CH-\overset{O}{\overset{\|}{C}}-OH} + \underset{\displaystyle R_2}{H_2N-CH-\overset{O}{\overset{\|}{C}}-OH} \xrightarrow{-H_2O} \underset{\displaystyle R_1}{H_2N-CH-\overset{O}{\overset{\|}{C}}}\underset{\text{peptide bond}}{-}\overset{H}{N}-\underset{\displaystyle R_2}{CH-\overset{O}{\overset{\|}{C}}-OH}$$

The peptide compound is termed di or tri- or tetrapeptide according to the number of amino acids rather than the number of peptide bonds. Thus a dipeptide contains 2 amino acids and a tripeptide contains 3 amino acids.

The peptide compound that contain 2-10 amino acid is usually termed short peptide and the peptide containing more than 10 (10-50) amino acid is termed long peptide or polypeptide while those containing more than 50 amino acids are usually called proteins. Physiologically active peptides include the following examples :

1. Glutathione : (GSH) :

Glutathione is atypical tripeptide formed of 3 amino acids, glutamic, cysteine and glycine.

glutamic -cysteine - glycine.

γ-glutamyl cysteinyl glycine

The active group of glutathione is the -SH group " thiol group " . Glutathione can donate hydrogen and change into oxidized form that can accept hydrogen to change to reduced form which is active.

$$2\ G\text{-}SH \xleftrightarrow[+2\,H]{-2\,H} G\text{-}S\text{-}S\text{-}G$$

Reduced form — Oxidized form

Glutathione is important for :

(A) Activation of many enzymes.

(B) Removal of the toxic H_2O_2 " hydrogen per oxide" that is normally formed in some metabolic reactions

$$H_2O_2 + 2G\text{-}SH \xrightarrow[\text{Selenium}]{\text{Glutathione peroxidase}} 2\ H2O + G\text{-}S\text{-}S\text{-}G$$

(C) Keeping the RBC's intact by reducing H_2O_2 and by decreasing the amounts of the methemoglobin in the RBC's .

(D) Inactivation of insulin hormone by breaking the (S-S) bonds between the 2 polypeptide chains of insulin.

(E)Conjugating toxic materials to reduce their toxicity prior to its excretion.

2. Angiotensin II :

Angiotensin II is a short peptide that contain 8 amino acids "octapeptide". It is produced as a result of hypotension or decrease in the serum sodium concentration, these changes causes the release of a proteolytic enzyme called Renin which act on $\alpha2$ -globulin.

Renin is secreted by cells near the glomerulus of the kidney.

$$\alpha 2\text{-globulin} \xrightarrow{\text{Renin}} \text{Angiotensin (10 amino acids)}$$

$$\text{Angiotensin I} \xrightarrow[\searrow 2\ a.a.]{\text{Converting enzyme}} \text{Angiotensin II}$$

Angiotensin II is a potent vasoconstrictor agent that leads to elevation of blood pressure. It also stimulates secretion of aldosterone hormone that causes retension of Na^{+} in blood and retension of water in the blood vessels to increase the blood pressure.

3. Vasopressin "Anti Diuretic Hormone"

It is a "nonapeptide" containing 9 amino acids. It is formed by the hyothalamus and stored in the posterior pituitary gland. Vasopressin "ADH" helps reabsorption of water in the renal tubules.

4. Oxytocin" 9 amino acids"

It is secreted from the hypothalamus and stored in the posterior pituitary gland as the ADH. Oxytocin is a hormone that stimulates controction of uterine muscle especially during labour.

5. Bradykinin

It is a nonapeptide "9 amino acid" produced in the blood. It is a strong vasodilator .

6. Polypeptide antibiotics :

They are produced by fungi. They contain both D and L amino acids. Examples are tyrocidin and gramicidin S.

Protein structure

Four levels of structure determine the final shape of the protein molecule "the three-dimensional struture ".

1. Primary structure :

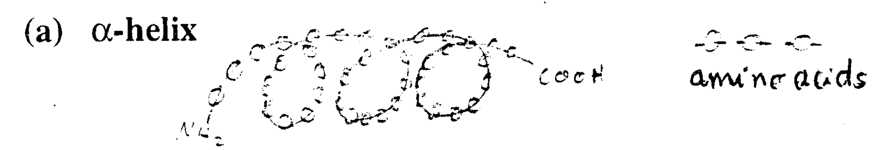
It is determined by the number, types and the sequence of amino acids in the protein molecule. The bond between the amino acids is the peptide bond that bind the amino acids within the polypeptide chain.

2. Secondary structure :

The polypeptide chain is not a straight chain.

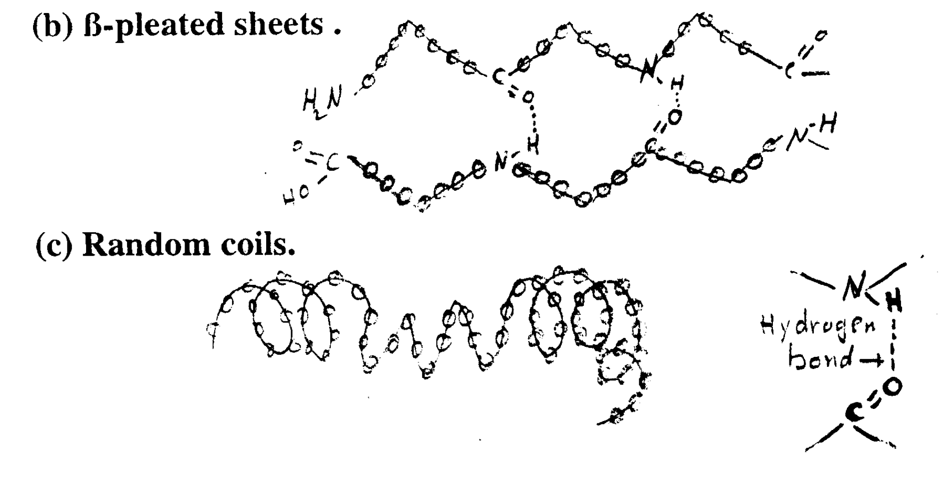
It is coiled on itself to form one of the following shapes :

(a) **α-helix**



The polypeptide chain is coiled or folded in clockwise direction " right handed". The folding is stabilized by hydrogen bonds and disulphide bonds (-S-S-).

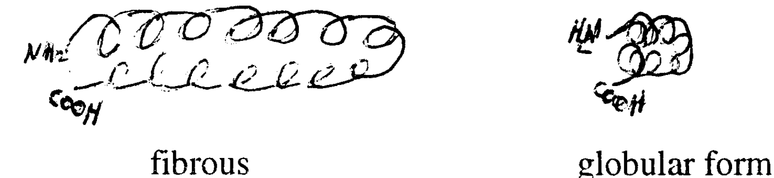
(b) ß-pleated sheets .



(c) Random coils.

(3) Tertiary structure :

It describes the relation of the segments of the coiled polypeptide chain to each other . The segments of the helix may be arranged in longitudinal layers to form fibres " fibrous proteins" or the segments are arranged in compact structure to form globules as in glubular proteins.



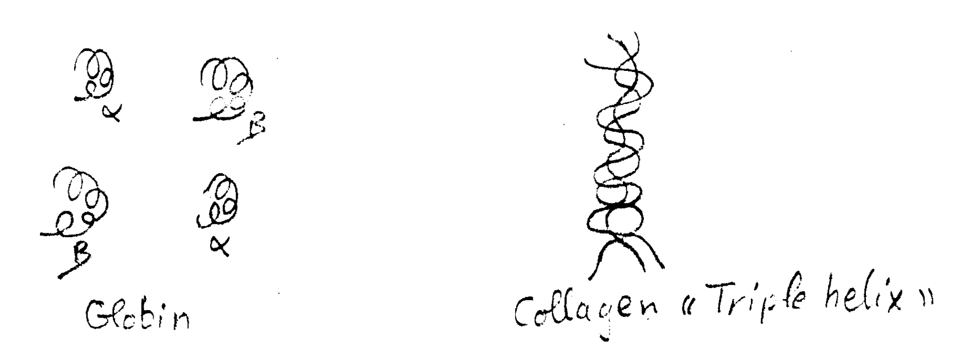
fibrous globular form

This structural level is stabilized by hydrogen bond (-C = O .... H - N-) , disulphide bond, [S-S-] ionic bonds between positively charged groups and negatively charged groups and and by hydrophobic forces.

4. Quaternary structure :

Some proteins are formed of more than one polypeptide chains " units" or " meres" linked together to form one molecule as Globin protein " 4 polypeptide chains" and collagen " 3 polypeptide chains".

The relation of units to each other to form the final shape of the molecule is called quaternary structure



Properties of proteins:

(A) Solubility .

Proteins differ in their solubility character. Albumin is soluble water , Globulins are soluble in dilute salt solution while scleroproteins are insoluble in most solvents.

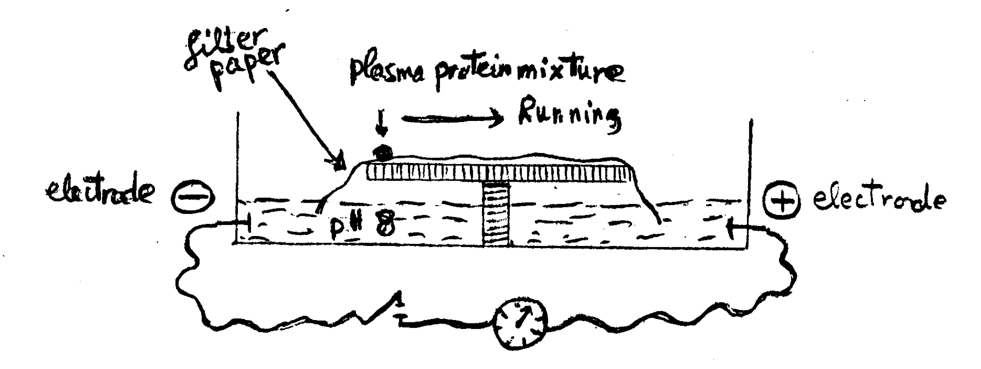
(B) Electric properties of proteins:

Each protein contains an amino group (-NH2) at the left end of its peptide chain and a carboxylic group (-COOH) at the right end of the chain. Threfore a protein molecule behaves in a similar way to a separate amino acid at different pHs.

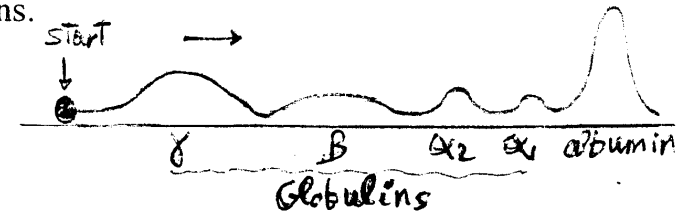
Proteins carry positive charge in an acid medium and a negative charge in an alkaline medium. At its specific isoelectric point (I.E.P) , the prtein becomes electrically neutral.

Electrophoresis is a method used for separation of proteins in a mixture as the serum proteins. The principle of such method depends on the fact that proteins carry a charge at alkaline or acid media and can then move in an electric field towards the opposite electrode. The velocity of movements of proteins in such electric field differs according to the size and shape of the molecule and the amount of charges carried by the protein molecule.

Plasma proteins and lipoproteins are separated by electrophoresis.



Serum proteins are separated into albumin, α_1, α_2, , ß, Y globulins.



(C) Denaturation of proteins:

Denaturation of protein molecule is the change of the protein from its native " natural" state to a disrupted or denatured form.The secondary, tertiary and if present the Quaternary strutures are lost due to cleavage of the bonds that sabilize them as hydrogen bonds, ionic bonds and hydrophobic bonds. The primary structure remains unchanged.

Factors that cause denaturation of proteins include:

(a) High temperature.

Cooking the meat by heating is important to facilitate digestion of its proteins because denaturation of meat proteins causes their coiled polypeptide chains to stretch to facilitate their digestion by proteolytic enzymes.

Fever "high body temperature " is dangerous because it can cause denaturation of our enzyme proteins.

(b) Very high or very low pH are important cause of protein denaturation.

Acidosis or alkalosis could be very dangerous due to the possibility of denaturation of our enzyme proteins.

(c) Radiations as X-ray and U.V. radiations and high pressure.

These factors are strong denaturating agents.

Effects of denaturation on proteins:

1. The solubility of the protein decreases and the protein precipitates.
2. The protein loses its biological activity. Enzymes, for example, lose their catalytic activity.
3. The non-covalent bonds that stabilize the protein structure are disrupted.

Protein classification :

(A) According to the structure, proteins are classified into:

[1] Simple proteins [2] conjugated proteins. [3] Derived **proteins.**

[1] Simple proteins

On hydrolysis , it produces amino acids only . Examples include.

(a) Albumin.

It is present in: - Plasma

- Milk (lact albumin)
- Egg white (ovoalbumin).

It coagulates on heating . It is easily dissolved in water.

(b) Globulins:

They exist in:

1. Blood plasma : α_1, α_2,ß- and γ-

- globulin in blood are also called immunoglobulin (antibodies)

2. Milk.
3. Eggs.
4. Small amounts in muscles (myoglobulin).

Globulin coagulates on heating. It is readily soluble in dilute salt solution.

N.B.: Albumin and globulins are proteins of high nutritional values because they contain all the essential amino acids in good amounts.

(c) **Scleroproteins** (Albuminoids) (fibrous). They are very resistant (strong) proteins. They resist hydrolysis . The cause is that they contain very large amounts of cysteine and other sulphur amino acids. They have many -S-S- bonds.

They support and protect the tissues.

Examples of scleroproteins include :

1. ***Keratin***:

   It is present in nails, hoofs, hairs, and wool.

   It resists hydrolysis due to its high content of sulphur.

2. ***Elastin***. It is present in elastic tissues as lungs and blood vessels.

(d) Protamines.

It is basic protein, rich in basic amino acids as arginine, lysine and histidine. It is present in connection with nucleic acid esp. in sperms.

(e) Histones.

It is a basic protein rich in basic amino acids. (Histidine).
It exists with DNA in the nucleus to form nucleoproteins.
Globin of Hb is a histone.
5 types exist in the nucleoproteins,
H-1, H-2a, H-2B , H-3 , H-4 (H=histone).

(f) Glutelins

They are present in wheat and rice . It is very rich in glutamic acid.

(g) Gliadins:

It is present in maize (Zein) and in wheat (gliadin) .They are very rich in proline.
Maize proteins (zein) is deficient in tryptophan and lysine.

[2] Conjugated proteins or (compound proteins)

The protein is formed of protein part (apoprotein) and non-protein part (prosthetic group).

Conjugated proteins include the following types :

(l) Glycoproteins (mucoprotein) (protein + carbohydrates) carbohydrates that may be present include :-

1. Mannose, galactose .
2. N-acetyl glucosamine.

N-acetyl galactosamine

3. Arabinose, xylose.
4. L-Fucose (methyl pentose)
5. Sialic acid (NANA)

Glycoprotein does'nt contain uronic acid (glucuronic acid)

Examples:

1. Collagen in connective tissue matrix .
2. γ -globulins (immunoglobulins).
3. Transferrin
4. Thyroglobulin (in thyroid follicles)

Collagen :

* It is the most abundant protein in body. It represents 30% of the total protein. It is the protein of the connective tissue .Its molecule is formed of 3 polypeptide chains "Triple helix" .
* It is an extracellular protein. It is present in the ground substance of connective tissue to support the nerves, vessels,...
* It is very rich in glycine. Every 3rd amino acid in its chain is glycine (X-Y-Glycine) . It is also rich in lysine and its hydroxy form, proline and hydroxy proline.
* It is synthesized in fibroblast as immature protein called pre-pro-collagen. It changes into procollagen then into mature collagen by certain changes in its structure as:

1. Hydroxylation of proline and lysine.

Prolyl-Hydroxylase

Proline ---------------------------> OH - proline

O2,Vitamin-C

Lysyl - hydroxylase

Lysine -------------------> OH - lysine

O_2,Vit.-C

N.B.:

Vitamin- C is necessary for the activity of th e hydroxylase . It protects the ferrous iron (Fe^{++}) in these hydroxylase from oxidation to ferric form (Fe^{+++}) which reduces the activity of the enzymes.

Vitamin - C

Fe^{+++} --------------------------> Fe^{++}

(ferric) (Oxidized form) (ferrous) (reduced form)

Deficiency of vitamin - C causes Scurvy. Manifestation of scurvy are due to the presence of weak immature collagen.

2. Glycosylateion:

Addition of glucose or galactose molecules into the OH group of hydroxylysine occurs within the polypeptide chains of procollagen, just before the formation of the triple helix.

3. Triple helix formation and cross linking of the collagen fibrils.

This change occurs outside the cell and results in association of the 3 separate procollagen chains to form the triple helix and to bind the collagen molecules together by covalent cross links in the connective tissue matrix.

Immunoglobulins "Ig"

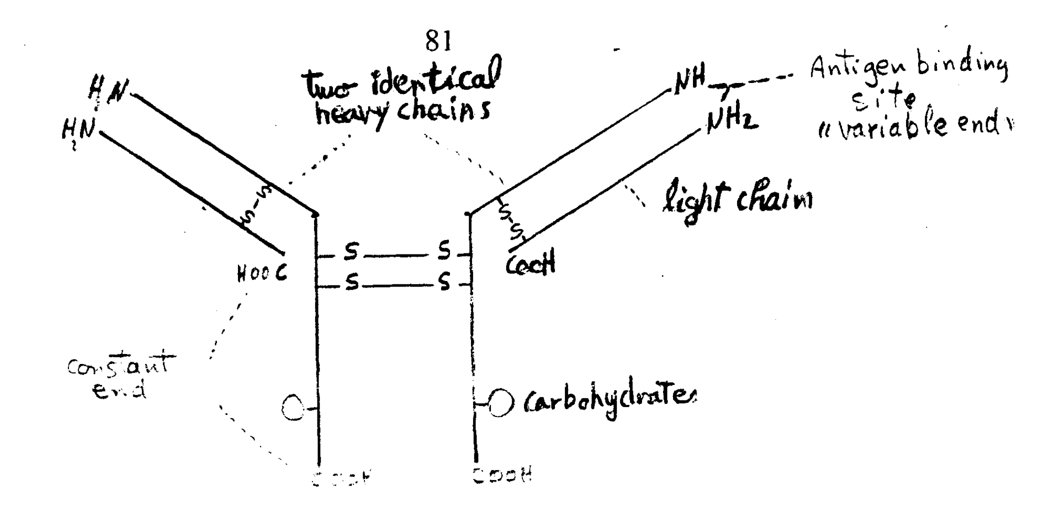
Immunoglobulins " antibodies" are mostly γ -globulins They are formed by ß-lymphocytes as a result of introduction of antigens into the inside of the body. Antigens ar foreign " non-self"compounds of high molecular weight, mostly proteins. Polysaccarides and some lipids as cardiolipin are alsoantigens. DNA is a weak antigen. When an antigen is introduced into the body through a cut in the skin or through the body orifices, it reaches the lymphoid system to be identified as a foreign agent by ß-lymphocytes which divide into clones of plasma cells. The plasma cells produce different antibodies "immunoglobulins" that bind specifically to that antigen and help its destruction by phagocytic cells.

Structure of immunoglobulins " antibodies"

Immunoglobulins are of five types based on their structure and function : IgG,IgA, IgM, IgD and IgE. Each type is formed of one or more of a basic unit. Each unit is a tetramer formed of 4 polypeptide chain, 2 identical long or heavy "H" chains and 2 identical light or short chains linked together by -S-S bonds.

The two short chains are either Kappa (K) or Lambda (λ) in all immunoglobulins.

The two long chains differ in the different immunoglbulins and their type determines the type of the immunoglobulin.



Structure of immunoglobulin unit

The antigen binding ends are variable in structure to adapt the antigens. The carboxylic end of the long and short chains are constant and contain small amounts of carbohydrate. This constant end is concerned with fixation of complements. The following table shows some characteristics of the different immunoglobulins.

Type of Ig	Heavy chain	Plasma concentration (g/L)	**Properties**
IgG	γ	14	Small in size being formed of one unit. It can cross the placenta. It is the major one in secondary immune response.
IgA	α	3.5	It is formed of 2 units. It is the main antibody of secretions as saliva, tears, sweat, mucus.

Type of Ig	Heavy chain	Plasma concentration (g/L)	**Properties**
IgM	μ (mu)	1.5	It is a pentamer, being formed of 5 units linked by S-S-bonds or J-chain . It is the major antibody in primary immune response.
IgD	(delta)	0.03	The function is not yet definitly known.
IgE	E(epsilon)	0.0-0.003	It is increased in allergic conditions

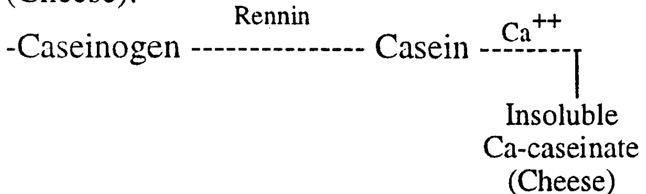
Bence-Jones protein:

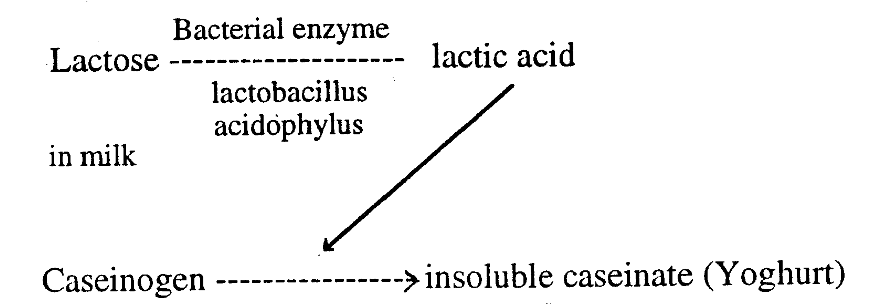
In plasma cell tumour "Multiple myeloma", the plasma cells produce many short chains and less long chains. The excess short chains are excreted in the urine . These short chains are called Bence-John's protein. This protein coagulates at 50°C and redissolves at 100°C.

Phosphoproteins:

*Protein with phosphates as the non-protein part.

* Caseinagen: It is the main milk protein .It is a soluble protein in the milk . It precipitates by changing into casein which forms with Ca^{++} calcium caseinate that precipitates taking the lipids with it (Cheese).





Caseinogen ---------------> insoluble caseinate (Yoghurt)

Nucleoproteins:

They are formed of DNA and histones or protamines. They form the chromatin materials or the chromasomes.

Lipoproteins:

They are proteins associated with lipid they serve transport of insoluble lipids (TG, phospholepids, cholesterol) in the blood.

Lipoproteins include different types as chylomicroms (1-2% protein, 90% TG,phospholipids), VLDL (Very Low Density Lipoprotein) LDL, HDL (High Density Lipoprotein) NEFA+ albumin (Non Esterified faty acid).

Metalloproteins:

Proteins associated with metals examples.

-Iron containing proteins as, Hemoglobin, myoglobin, transferrin , ferritin and cytochromes.

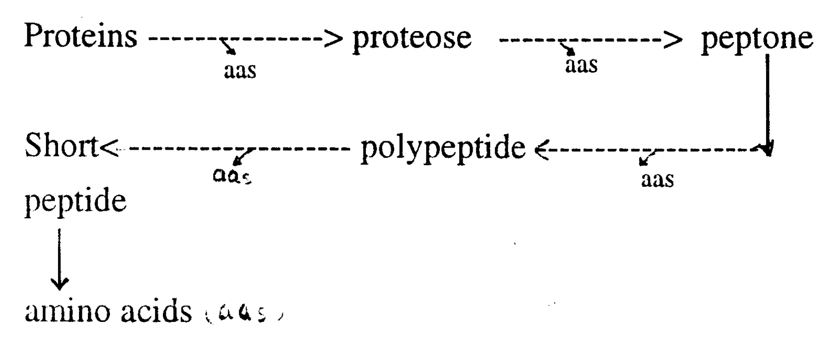
-Copper containing proteins as ceruloplasmin(ferrooxidase) and tyrosinase.

-Magnesium containing protein as Kinases, Phosphorylase and carboxylase.

- Zinc containing proteins as Insulin and carbonic anhydrase.

3-Derived proteins:

*They are the products of hydrolysis or digestion of other proteins.



* Denatured proteins are also derived protein.

(B) <u>**Nutritional classification of proteins:**</u>

Proteins are classified according to their nutritional value into :

1. Proteins of high nutritional value.

They are characterised by their high content of essential amino acids. They are mostly of animal origin as proteins of the milk, egg, meat ,fish.

2. Proteins of low nutritional value.

They are deficient in one or more of the essential amino acids. They are mostly of plant origin ,e.g. Maize protein is lacking tryptophan.

Hemoglobin (Hb)

It is a globular protein present in high concentration in the red blood cells. Being a chromoprotein, it confers its red color to the blood . Its concentration in the blood ranges between (12-18 g/dl) depending on age and sex. It is formed in immature erythrocytes in the bone marrow. It transports oxygen from the lungs to the tissues and carries CO2 and protons to the lungs.

Structure :

Hemoglobin belongs to the class of conjugated proteins. Each molecule is formed of 4 heme units + globin (4 polypeptide chains).Heme is a ferrous protoporphyrin.

M = methyl ($-CH_3$)

V = Vinyl ($-CH = CH_2$)

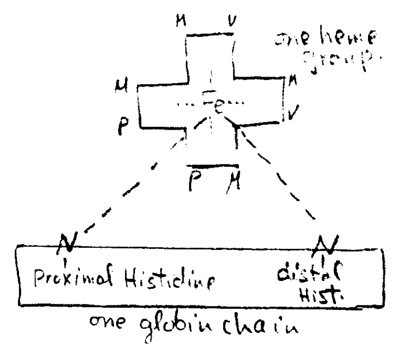
P = Propionic ($-CH_2\ CH_2\ COOH$)

Porphins are cyclic compounds formed of 4 pyrol rings linked by methenyl bridges (-CH=) . When the positions indicated by the numbers are substituted according to the above diagram, it forms protoporphyrin, other types of porphrins are formed by different substitutions.

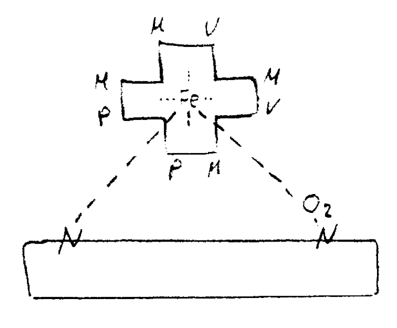
As stated above heme is a ferrous protoporphirin (IX) . It is the prosthetic group attached to each one of the four polypeptide chains of globin, i.e. , there are four heme groups in each hemoglobin molecle. Each heme group contains an iron atom in its center . The iron atom is in the ferrous state (Fe^{++}) in the funtionally effective hemoglobin (and myoglobin)

The ferrous atom in the heme can form five or six ligand bonds, depending on whether or not O_2 is bound to the hemoglobin. Four of these bonds are to the pyrrole nitrogens of the protoporphyrin. The fifth bond of the ferrous atom of each of the hemes is to the nitrogen of an imidazole group of histidine (in each chain of globin) this is called proximal histidine.

In oxyhemoglobin, one oxygen molecule (O_2) forms a sixth bond to the ferrous atom in each heme group. In this form, the O_2 is placed between the ferrous atom and a second histidine imidazole called the distal histidine. In the deoxyhemoglobin the O_2 binding position of the ferrous atom is unoccupied.



In deoxyhemoglobin form.



In oxyhemoglobin form.

Globin is a protein formed of four polypeptide chains arranged in 2 different pairs. The two chains of each pair have the same primary structure which differs from that of the other pair.

The common form of human adult hemoglobin, HbAl (HbA) has two α chains and 2 ß chains and its polypeptide chain composition is therefore α_2 β_2. Each α-chain contains 141 amino acids and the ß-chain contains 146.

In the α-chain the heme iron is linked covalently to the imidazol nitrogen of a histidine amino acid at position 87 (proximal histidine).

In the ß-chain this proximal histidine lies at position 92 of the N-terminal end of the polypeptide chain.

Two other chains (γ & δ) replaces ß-chain in hemoglobin F and A_2 respectively, which occur as a minor components in the normal adult hemoglobin . The two chains differ from ß-chain in the amino acid sequence.

Normal Human Hemoglobins

In the course of a lifetime humans synthesize different hemoglobins (different globins). All these hemoglobins contain 2 α-chains and differ in the structure of the other 2 chains.

The normal human hemoglobins include :-

[1.]Hemoglobin A (Al) .

It makes up about 95% . Its globin structure is α_2 β_2.

[2.]Hemoglobin A2.

It makes up about 2.5% of the total.Its globin structure is $\alpha 2 \delta_2$ Its level increases in ß-thalassemia and megaloblastic anaemia. It decreases in iron deficiency anaemia.

[3]Hemoglobin A3 (glycosylated hemoglobins). (HbA1C)

This type of hemoglobin was first discovered as a minor component very close to the main hemoglobin A on starch gel electrophoresis. Subsequently, using a better method of separation(cation-exchange resins chromatography) it was found to be formed of several minor components called A_{1a}, A_{1b} and A_{1c}. Very little is known about the structure or function of hemoglobins A_{1a} and A_{1b}. Hemoglobin A_{1c} is the most abundant derivative.

Hemoglobin A_{1c} (Glycosylated hemoglobin).

It is formed as a result of condensation of glucose with the amino terminal valine in the ß-chain of hemoglobin A. This glycosylation reaction is a nonenzymatic one. The reaction is slow and irreversible. The aldehyde group of glucose condenses with NH_2 group of the terminal amino acid in the ß-chain to form a shiff base (aldimine) which undergo rearrangement to form a more stable ketoamine.

CHO
H C OH
HO C H
H C OH
H C OH
CH2OH
glucose

\+ β-chain of globin (NH_2 – protein) ⟶

CH=N-protein
H C-OH
HO-C-H
HC-OH
HC-OH
CH2OH
Aldimine

Glycosylation reaction takes place continuously during the life span of the red blood cell. The degree and duration of hyperglycaemia affects the amount of glycosylated hemoglobin in the blood. Considering the life span of normal erythrocytes (120 days) , an elevation of glycosylated hemoglobin, reflects elevation of blood glucose level over the previous 2-3 months. Therefore the level of HbAlc is a good indicator of the degree of control of the blood glucose level.

Normal level of HbA_{1c} =5 - 9 % of total Hb

In diabetes mellitus = > 9% of total Hb.

In well controlled diabetes the level may be within normal.

In poorly controlled diabetes the level of HbA_{1c} is high, 18% of total or even more. Therefore it is used mainly for follow up

The process of hemoglobin glycosylation is a post-translational modification which increases during sustained periods of hyperglycaemia. In a similar way other proteins like the lens protein α-crystallin, proteins in the red cell membrane and the cell wall of capillaries may be glycosylated and this may contribute to the medical complications caused by diabetes like cataracts, nephropathy, neuropathy and coronary heart disease.

Determination of HbA_{1c}

I. A red blood cell hemolysate is chromatographed on an ion exchange column. The eluate is measured spectrophotometrically at 410 nm.

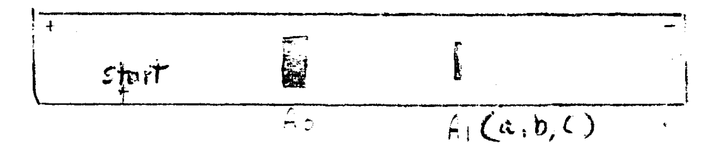
In this method hemoglobin is separated into:

HbA = 93.4% of the total Hb.

HbA_{1a} + HbAlb = 2.4% of total (1.6% + 0.8%).

HbA_{1c} = 4-8.5%

II. Electrophoresis on agar gel.



2 bands are separated.

HbAl (A_o) band 93.4%

HbAl (a,b,c). 6.6%

[4] Hemoglobin F : - It is present in the blood of the foetus.

* The Globin chain composition is $\alpha 2 \gamma 2$.

* It has higher affinity to O_2 than the HbAl in mother's blood.

* It make up 2-2.5% of total Hb in adults.

* Its concentration starts to decrease in the blood of infants during the first 6 months after the delivery .

*It increases in thalassaemia.

ENZYMES

Definition:

- Enzymes are protein catalysts for chemical reactions . They are produced by the cells.

- A catalyst is a substance that accelerates a chemical reaction without being consumed in the reaction . They remain unchanged at the end .

- Enzymes are produced in the cells but they can act outside the cells too.

- Enzymes are specific. Each enzyme catalyses one type of reaction and acton one substrate or intimately related substrates. Chemically the enzymes are classified into:

1. Simple proteins e.g. lipase ,amylase and protease.

2. Conjugated proteins being formed of :

A. Protein part: apoenzyme .

B. Non-protein part: coenzyme "Prosthetic group or cofactor"

The prosthetic group is tightly bound to the apoenzyme and mostly is a metal. The cofactor is mostly of vitamin origin and is loosly bound to the apoenzyme.

The whole enzyme "apoenzyme + coenzyme " is called the holoenzyme.

*All enzymes are protein in nature except some recently discovered ribozymes, which are RNAs that catalyse their own splicing.

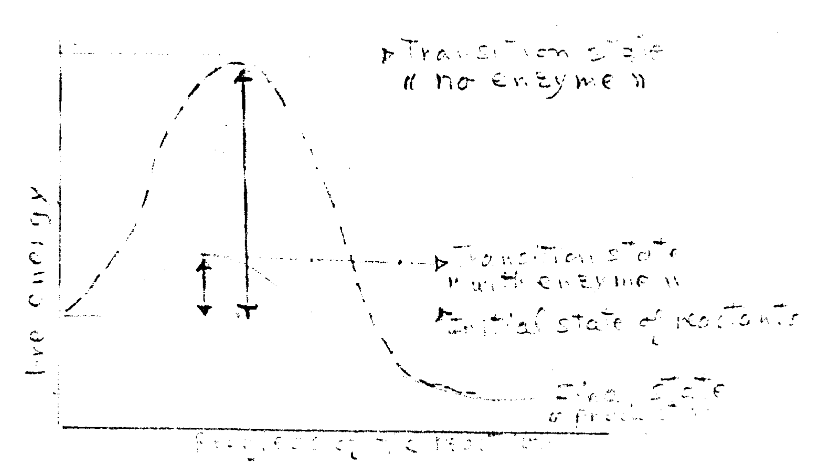
Mechanism of Action of Enzymes:

* Enzymes speed up the reactions. They are the most efficient catalysts. They increase the rate of the reaction by 10^{20} times over the uncatalyzed reaction.

N.B.: Non-enzymatic catalysts enhance the reaction by a factor of 10^2-10^4.

How do enzymes work ?

*Enzymes reduce the activation energy of a reaction which is the energy required to initiate the reaction or the amount of free energy (ΔG^o) required to bring the reactants to the transition state (the maximum of the curve).



The substrate "S" at the transition state bind to certain groups in the catalytic "active" site of the enzyme "E" to form enzyme-substrate complex E-S. complex. The energy derived from E-S formation is called binding energy which is the major source of free energy that is used by the enzyme to lower the activation energy of the reaction.

E+S--------> E-S complex -------> E-product---> E+product.

The enzyme catalytic site identifies the specific substrate by being complementary with the shape of the substrate molecule. This is the basis of enzyme specificity which could be explained by two models.

1. Key and lock model.

The substrate fits to the binding site just as a key fits into its proper lock. This model is a rigid one.

2. Induced fit model.

The binding active site of the enzyme changes its shape to fit the substrate . This model is flexible.

Key + lock

induced fit

Enzyme specificity:

Enzymes are highly specific. They are able to catalyse one specific reaction or a group of closely related reactions. The enzyme specificity is exhibited in various forms. The degree of enzyme specificity is variable as in the following :

1. Absolute specificity :

The enzyme acts on one substrate only . Example

Glucose oxidase acts on glucose only, uricase acts on uric acid urease acts on urea only.

2. Relative specificity:

The enzyme acts on a group of closely related substrates.

e.g. Lipases act, on all triglycerides

Hexokinase acts on all Hexoses.

3. Optical specificity:

When two isomers of a substrate exist, enzymes act on one isomer only.

* L-amino acid oxidase act on L-amino acid. D amino acid oxidases acton D-amino acid.

* Enzymes of glycolysis act on D-sugars.

4. Dual specificity:

Enzymes act on two different substrates e.g. Xanthine oxidase acts on both Xanthine and hypoxanthine

Hypoxanthine --X-oxidase--> xanthine --X-oxidase--> Uric acid

5. Group specificity:

The enzyme needs the presence of certain group in the substrate e.g.

*Trypsin splits the peptide bonds involving the carboxylic group of lysine or arginine "basic aa."

* Thrombin splits peptide bonds formed of -COOH of arginine and NH_2 of glycine.

* Pepsin beaks down peptide bonds having aromatic acids.

Enzyme terminology (Nomenclature):

* Early attempts of enzyme terminology and classification used their old names like pepsin, trypsin, ptyalin. Although these old name are not informative, still they are used:

* Enzymes were later named according to their substrate after addition of the suffix-ase.

e.g. lipase , protease, ureas, uricase.

*Enzymes were named in groups according to their activity

e.g. oxidases, dehydrogenases , hydrolases, phosphorylases.

In this type of terminology the related name is preceeded with the name of the substrate.

e.g. glucose oxidase, lactate dehydrogenase.

Eznyme classification:

Enzymes are classified, according to the type of the reactions they catalyse, into 6 major classes.

Class No. Class Name

1-Oxido-reductases 2. Transferases

3. Hydrolases 4. Lyases

5. Isomerases 6. Ligases or synthetases or synthases

According to the international union of biochemistry (IUB) system, each enzyme has a code number formed of four digits .

The first digit = the class

The second digit = subclass. It indicates the donated group

The third digit = subsubclass. It indicates the acceptor.

The fourth digit = The enzyme name.

e.g. An enzyme code (E.C.) of 2.7.1.1 denotes

class 2 = Transferase

Subclass 7 = Transfer of phosphate.

Subsubclass 1 = An alcohol is the acceptor of phosphates

1 = Hexokinase

Examples of each class will be studied during the study of metabolism.

Factors affecting the enzyme activity:

Enzymatic activity is affected by the following factors :

1. Temperature :

Fig. (1) represents the rate of enzymatic reaction measured at different temperatures. Each enzyme has an optimal temp. at which its activity is maximum.

For most enzymes, their optimal temperature are at those of the cells in which they occur. For animal enzyme the optimal temp. is 37°C -40°C.

At the zero degree the enzymatic activity is stopped. The enzyme is not destroyed and it still retains its activity.

Increasing the temperature leads to rise in the rate of enzymatic activity until a maximum at the optimal temperature. The rise in

temperature increase the kinetic energy of the reactants. This allows the reacting malecules to come in contact more frequently. With further rise of the temperature more than the optimum temperature, the enzymatic activity drops rapidly due to denaturation of the enzyme protein.

2. Effect of pH

*The pH affect the ionic state of the enzymes and the substrate

*Each enzyme has an optimal pH at which its activity is maximal. This is because the ionic state of the enzyme at this pH is most suitable for increasing the effective concentration of the enzyme and substrates.

Pepsin........................ optimal pH is 1.5

Pancreatic lipsase....... optimal pH is 8

Most of the enzymes in the cell act at pH between 4-8 the optimum pH for these enzymes is 7.3°C.

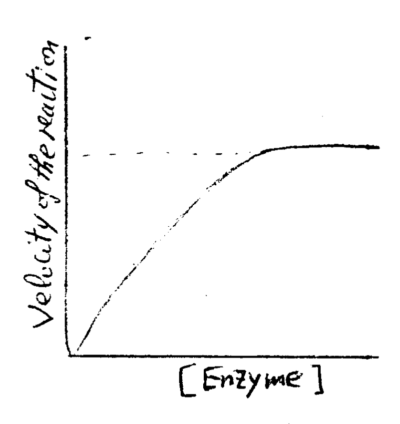
At extremes of pH away from the optimum pH, the enzyme looses its activity due to :

1. denaturation.

2. decreased effective concentration.

3. Enzyme concentration:

Keeping all other factors constant, the increase in enzyme concentration leads to increase in the velocity of enzymatic reaction until equilibrium occur. At equilibrium further increase in enzyme concentration will not increase the velocity.



4. Substrate concentration

Enzyme kinetics:

The increase in the substrate concentration leads to increase in the velocity of the reaction until a maximum velocity is attained at equilibrium.

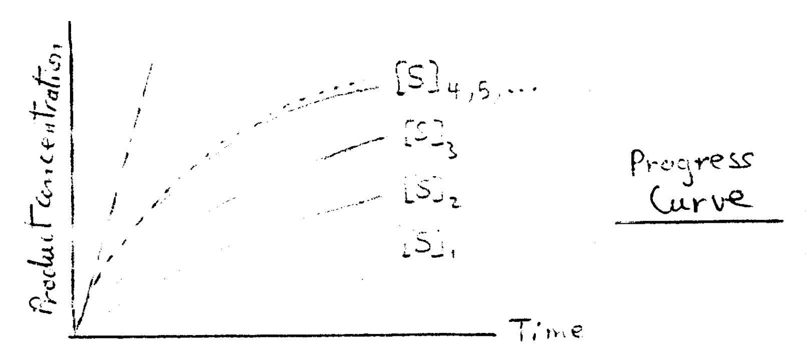
Kinetics is the study of the rate of the change of the initial state of the reactants and products to the final state.

The change of reactants can be expressed as velocity or rate of enzymatic reaction . Both words (velocity and rate) were usually used interchangeably. Velocity is often used.

Velocity --------> mean the change in the concentration of substrate or product per unit time.

Rate ---------> means the change of the total quantity (moles, grams) of substrates or products per unit time.

The velocity of a reaction can be determined from a progress curve where the product appearance is plotted against time.



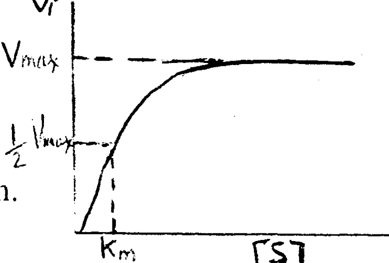
The initial velocity V_i of a reaction is the velocity at the early begining of the reaction when the product is negligible.

For the given reaction, $S+E \underset{V_2}{\overset{V_1}{\rightleftharpoons}} P + E$

$Vi = V_1 - V_2$ when V_2 is almost zero.

The initial velocity of the reaction (Vi) is determined from the slope of the curve at the beginning of the reaction. Initial velocity can be determined for different substrate concentrations ([S]1 -- [S]4) . Increasing the substrate concnetration increase the initial velocity of the reaction.

A substrate saturation curve can be done by plotting the initial velocities against their corresponding substrate concentrations. Michael's constants K_m and V_{max} can be obtained from that curve.



V_{max} is the velocity obtained at enzyme saturation

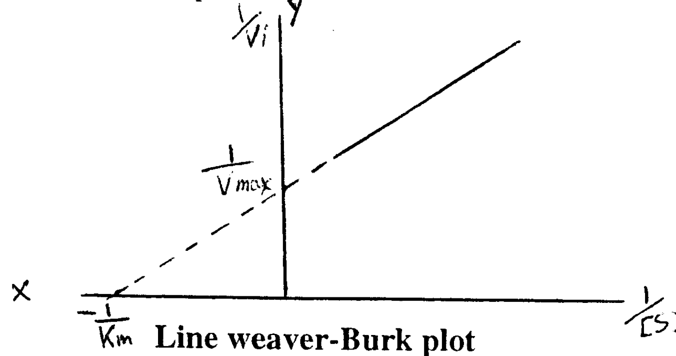
K_m is the [S] that produces half the maximum velocity of reaction.

K_m and V_{max} are two constants specific to each enzyme under standard conditions. Km denotes the affinity of the enzyme for substrate.

If K_m is high the affinity is low ?

If K_m is low the affinity is high ?

K_m determination from substrate saturation curve is not very accurate. It can be accurately determined from Lineweaver-Burk plot where the reciprocals of the initial velocities ($1/V_i$) are plotted against the reciprocals of the substrate concentration.



Line weaver-Burk plot

A line is yielded. Its intercept with Y axis is $1/V_{max}$ and with X-axis is $-1/km$.

5. Enzyme stimulation:

Stimulation of enzyme activity can be done in many ways:

(1) Stimulation of enzyme by changing the conformation of the enzyme molecule so that the groups of the active site ".OH,SH and imidazol group" become more close to each other.

Examples:

-Cl^- is stimulator to α- amylase .

-Ca^{++} is stimulator to thrombin.

(2) Reversible covalent modification or addition or removal of phosphate group to the enzyme protein can cause stimulation, e.g.

(a) Phosphorylation of glycogen phosphorylase causes its activation. Phosphorylase kinase enzyme adds the phosphate group to the phosphorylase molecule.

-C-AMP stimulates the synthesis of phosphorylase "protein" kinase .

-Anti-insulin hormones "as glucagon adrenaline, cortisone, thyroxine." stimulate the synthesis of C-AMP from ATP.

$$ATP \xrightarrow{\text{adneylate cyclase}} C\text{-}AMP$$

(b) Removal of phosphate = de-phosphorylation from the protein enzyme may cause its stimulation, as Glycogen synthase is active in dephosphorylated from. A phosphatase removes the phosphate from the glycogen synthase causing its activation. Insulin hormone stimulates the phosphase and decreases the C-AMP level which inhibits the glycogen synthase by promoting its phosphorylation.

(3) Proteolytic cleavage :

Cleavage or removal of part of the inactive enzyme " proenzyme or zymogen" to form the active enzyme is seen clearly in the digestive enzymes as pepsin and trypsin which are produced as zymogens called pepsinogen and trypsinogen respectivity. Blood coagulation cascade of enzymes are another example. Proteolytic cleavage produces a rapid activation of the enzyme .

(4) Induction of enzymes

Induction is the synthesis of an enzyme in the presence of an inducer substance. The inducer may be the substrate itself or a drug.

6. Enzyme inhibition:

2 main types.

(A) Irreversible inhibition:

The enzyme activity is lost. The main causes include :(a)Denaturation of the enzyme protein as by X-ray, heat, extremes of pHs

(b) Enzyme poisons as the heavy metals "Hg, Ag, Zn". They form covalent bonds with groups in the active site.

(B) Reversible inhibition:

The enzyme activity is inhibited but it can be regained after removal of the inhibitor from the medium. 2 main types are discussed .

1. Competitive inhibition:

*The inhibitor (I) is very similar to the substrate of the enzyme (S) in structure.

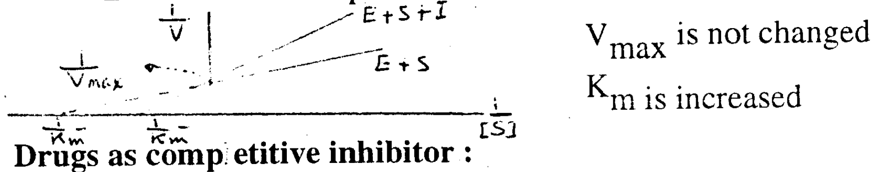
* Both the (I) and the (S) compete for the active " catalytic" site of the enzyme (E).

E + S ----------> E-S-complex ----------> product +E

E +I -----------> E-I complex -------X-----> No product

* High concentration of (S) dislodges the (I) from its binding to the active site of the enzyme and the activity of the enzyme is regained.

* The effect of such inhibition on V_{max} and K_m is shown by Lineweaver-Burk plot.



V_{max} is not changed

K_m is increased

Drugs as competitive inhibitor :

Some drugs act by inhibition of certain enzymes competitively as:

1. Allopurinol " xyloric"

This drug is used for the treatment of hyperuricaemia "Gout". It decreases the synthesis of uric acid by competitive inhibition of the Xanthine oxidase enzyme (X-oxidase)

Hypoxanthine --(X-oxidase)--> Xanthine --(X-oxidase)--> Uric acid

2. Sulphonamides:

They inhibit the growth of bacteria because they inhibit the synthesis of folic acid from Para Amino Benzoic Acid (PABA) in the bacterial cell.

PABA --(Enzyme)--> folic acid

Folic acid is needed for the growth of bacteria. Sulphonamides are similar in structure to PABA

3. Methotrexate

It is a drug used in the treatment of Malignant tumours " chemotherapy, cytotoxic drug".

Tumour cells need tetrahydrofolic acid (THF) for the synthesis of thymidine nucleotide and DNA during cell division.

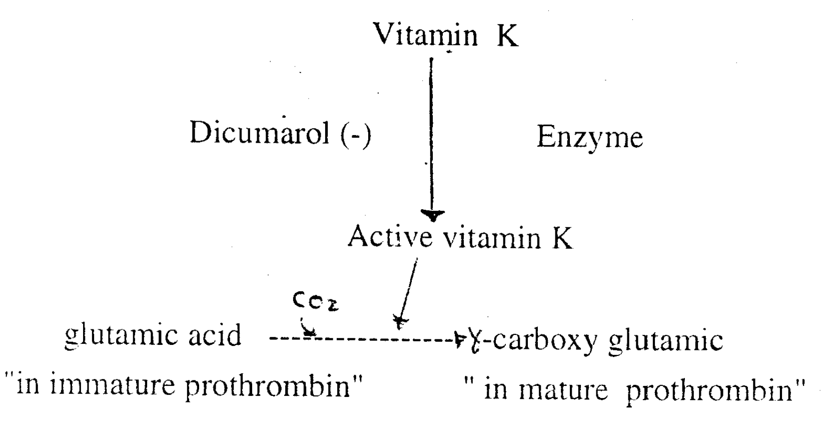
THF is regeneratred from dihydrofolate through the following reaction:

Dihydrofolate ---------------------reductase---------------------> THF

Methotrexate is similar to dihdyrofolate i structure , so it inhibits the dihydrofolate reductase.

4. Dicumarol

It is an anticoagulant drug that is taken orally . It inhibits the formation of the normal prothrombin by inhibiting the activation of vitaminK needed for synthesis of normal prothrombin "mature".



The antidote for dicumarol overdosage is vitamin K.

2. **Non-competitive Inhibition:**

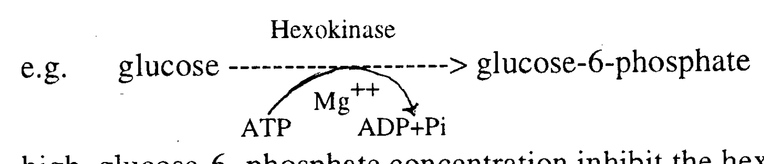
*The substrate (S) and the inhibitor (I) are **not** similar in structure.

* The inhibitor is **not** attached to the active site of the enzyme. The inhibitor is bound to other site "allosteric site". N.B. allo=other

* Such binding causes conformational changes in the enzyme molecule so that the groups in the active site get away from each other leading to inhibition.

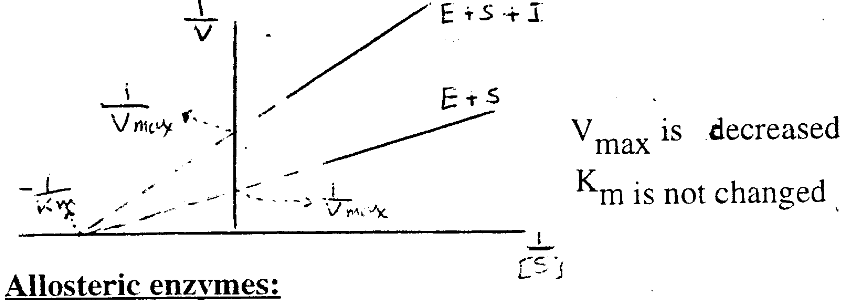
*Removal of the inhibitor causes the enzyme activity to be regained.

*The inhibitor is mostly a metabolite in the enzyme pathway. This is the bases of the feed-back control of the enzyme activity where the product of an enzyme inhibits the enzme " allosteric inhibition" when its concentration is high.



high glucose-6- phosphate concentration inhibit the hexokinase.

* The effect of non-competitive inhibition on V_{max} and K_m is shown in the following figure.



V_{max} is decreased

K_m is not changed

Allosteric enzymes:

* They are enzymes whose activity may be modulated (affected) by the presence of an allosteric effector attached to an allosteric site.

* They include the enzymes whose catalytic activity is regulated by feed-back control. They have allosteric " other sites" sites which is different from the catalytic sites. They generally catalyse irreversible reaction. They are large enzymes having mor than one unit .

*Allosteric enzymes exhibit sigmoidal substrate saturation kinetics.

- In absence of allosteric inhibitor, the curve is hyperbolic one.

-In the presence of allosteric inhibitor, the curve is sigmoidal. This, indicates the presence of 2 or more catalytic sites in an enzyme.

In this case,binding of onesubstrate at a catalytic site, facilitates binding of the second substrate at the second catalytic site and so on.

This is called cooperative effect

(it is similar to binding of O2 to hemoglobin.

Michael mentin kinetics do not apply to them because the relation between [S] and velocity produces sigmoidal curve.

Coenzymes:

they are organic molecules , often but not always derived from a vitamin, which are essential for the activity of many enzymes . They are loosely attached to the enzyme. They are characterised by being non protein and heat stable.

Reactions that require coenzymes include:

1. Oxidoreduction reaction - class (1)
2. Group transfer reaction - class (2)
3. Isomerisation reaction - class (5)
4. Synthetase (ligases) reaction - class (6)

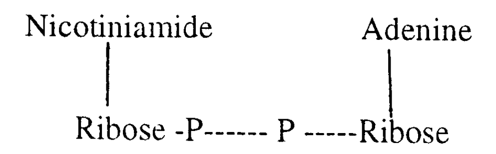
Classification

According to the group transferred by the coenzyme they are classified into:

A. **Coenzymes for transfer of hydrogen (hydrogen carrier) this group includes.**

-CoI = NAD

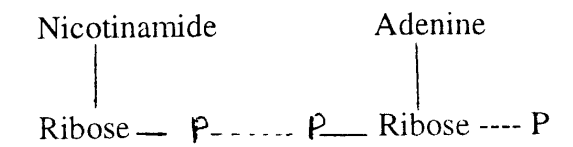
Nicotinamide Adenine dinucleotide



Nicotinamide is derived from the vitamin " Nicotinic acid"

2. CoII = NADP

Nicotinamide adenine dinucleotide phosphate



3. CoIII = Nicotinamide - Ribose - P

Nicotinamide -Mono-Nucleotide NMN

4. FMN = Flavine mononucleotide

flavine-Ribitol-P. Vit. ß2 (riboflovin) is Flavin-Ribitol.

5. FAD = Flavin adenine dinucleotide

Flavine
Ribitol------ P ---- P ----- Ribose

Adenine (attached to Ribose)

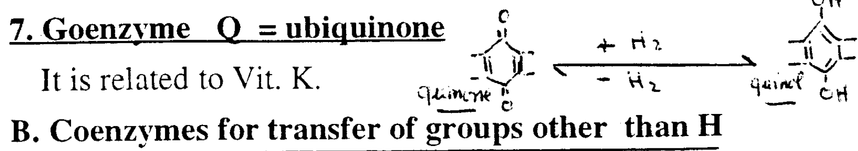
6. **Lipöic acid:**

L(—SH)(—SH) $\underset{+2H}{\overset{-2H}{\rightleftharpoons}}$ L(—S)(—S) (S–S bridge)

Lipoic acid is a vitamin of the B-complex

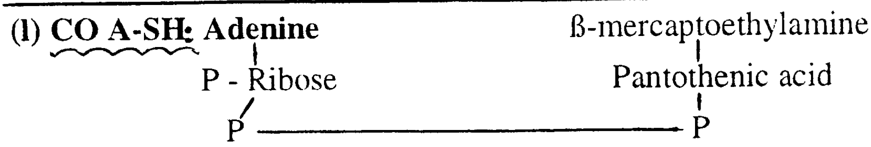
7. Goenzyme Q = ubiquinone

It is related to Vit. K.



B. Coenzymes for transfer of groups other than H

(1) CO A-SH: Adenine



COA-SH is an acid carrier. It carries the acyl group as acyl-COA .e.g. acetyl COA. Patothenic acid is a vitamin of the B-complex.

(2) Thiamine Pyrophosphate(TPP):

It is derived from thiamine "Vitamin B_1" . It is important in the following reactions:

(1) Decarboxylation (oxidative type) of α-ketoacids e.g. oxidative decarboxylation of pyruvate to form acetyl COA.

(2) Transfer of ketol group by transketolase enzyme in the Hexose Monophosphate pathway. These two reaction are very important in carbohydrate metabolism.

(3) Pyridoxal phospate (PLP)

It is derived from Pyridoxine "Vitamin B_6". It is important in :

(1) Transamination reaction. "GPT or GOT" to carry the NH_2 group.

(2) Decarboxylation of amino acids to form the corresponding amine. Histidine ---PLP, CO_2---> Histamine .

(4) Cobamide "derivative of Vitamin-B_{12} " " cobalamine"

it is important in transmethylation reaction.

Homocysteine ---CH_3, B_{12}---> Methionine

(5) Folate (F) as FH_4 " Tetrahydrofolate". The folate is a vitamin. Folates carry one carbon fragment as formyl and methenyl groups for purine nucleotide biosynthesis.

(6) Biotin = Cocarboxylase:

biotin is a vitamin. It is important for addition " fixation" of CO_2 in the carboxylation reaction.

e.g. pyruvate + CO2 + ATP ---Carboxylase, Biotin---> oxaloacetic acid + ADP + Pi

Isoenzymes (isozymes):

Isozymes are different molecular forms of an enzyme. They all possess identical enzymatic activity, (they catalaze the same reaction), but they differ in the following:-

(1) The chemical structure of their molecules. The structure of the active site is the same for all isozymes but the amino acid sequence in many parts of the peptide chain may differ considerably.

(2) The physical properties are different due to differences in the chemical structure.

* The isoenzymes migrate differently due to differences in the chemical structure .

  The isoenzymes migrate differently on electrophoresis.

* Some isoenzymes are more heat stable than the others, e.g. LDH-l is more heat stable than LDH-5.

(3) The antigenic properties are different especially for the isoenzymes that show marked differences in the structure.

e.g. LDH-l and LDH-5, placental alkaline phosphatase from other alkaline phosphatases (intestinal, hepatic, bone).

(4) The isoenzymes of a given enzyme are present in different concentrations in the different tissues., g.e. LDH-l is present in higher concentration in the heart muscle, while LDH-5 predominates in the liver and muscles. This phenomena is utilized in diagnosis of the diseased tissues.

(5) Isoenzymes may show differences in their relative specificity to certain substrates e.g. LDH-l is more active with ß-hydroxy butarate as substrate than is LDH-5.

On the other hand , isoenzymes are all similar in catalysing the same reaction. This is due to the structural similarity of their active sites.

Examples of isoenzymes used in clinical diagnosis :

1. **LDH isoenzymes** 5 isoenzymes are detected by electrophoresis.

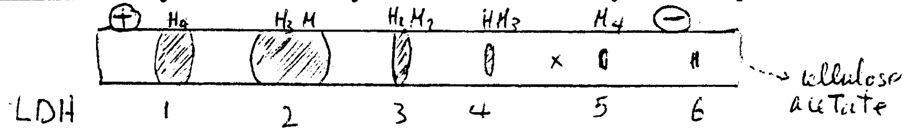


Fig. : Electro-phoretic pattern of serum LDH-isonezymes on cellulose acetate.

The LDH-isoenzymes are proteins formed each of four subunits (tetramers). The fours subunits of the isoenzyme separated from the heart are all identical and designated (H) units. Thus the LDH isoenzyme of the heart is formed of four H units and called LDH-1.

On the other hand the four subunits of the isoznyme of the liver and muscles are all identical and designated (M) units. The LDH isonenzyme of the liver or muscles is formed of 4 M units and called LDH-5.

The other LDH isoenzymes are formed each of a mixture of heart (H) muscle (M) units.

LDH-1	HHHH	44.5% of total
LDH-2	HHHM	46.0% of total
LDH-3	HHMM	3.8% of total
LDH-4	HMMM	1.0% of total
LDH-5	MMMM	0.5% of total

Predominant elelvation of LDH-1 & 2 occurs in myocardial infarction and renal damage.

Predominant elevation of LDH-2 and 3 occurs in acute Leukaemia and malignancies.

Predominant elevation of L. H-5 occurs in damage of liver or muscle tissues.

N.B.: Recently LDH-6 has been identified.It reflects liver injury secondary to severe circulatory failure.

(2) **Creatine kinase isoenzymes:**

Creatine kinase enzyme (CK) is found in the heart muscles, the brain, skeletal muscles and smooth muscles.

It catalyses the reaction.

$$\text{Creatine phosphate} + \text{ADP} \overset{\text{CK}}{\rightleftharpoons} \text{creatine} + \text{ATP}$$

The (CK) isoenzymes are three in number, each one is formed of 2 subunits (polypeptide chains).

The 2 subunits found in muscle tissue are identical and designated as M units . Therefor the structure of the muscle CK-isoenzyme is (MM).

The 2 subunits found in the brain and smooth muscles are identical (B units). The structrure of the brain & smooth muscle CK-isoenzyme is (BB) .

The cardiac muscle also contain an isoenzyme formed of the two subunits (M.B) this MB form of CK is normally undetectable (very low) in the plasma. It's level increase in myocardial infarction.

CK-MM level in the blood is elevated in myopathies.

CK-MB is elevated in myocardial infarction.

CK-BB is elevated in brain diseases.

PLASMA ENZYMES IN DIAGNOSIS

Enzymes in the plasma are of two types:-

(A) Plasma specific enzymes . They have a definite function in the plasma. They are present at higher concentrations in plasma.

Examples:

Lipoprotein lipase.

Plasmin

Thrombin

Ceruloplasmin " ferroxidase"

(B) Non-plasma specific enzymes

They have no function in plasma. They are present normally at higher concentration inside the cells. Their levels increase in plasma due to damaged of their tissue origin , as in inflammation or trauma.

They include :

1. Enzymes of secretions as amylase, lipase, pepsin.
2. Metabolic enzymes. They are originally present in the cytoplasm, cell organells or in the membrane.

Examples:

Lactate dehydrogenase (LDH) in cytoplasm

Acid phosphatase (ACP) in lysosomes

Alkaline phosphatase in membranes

Cholesterol esterase in Ribosomes

Amylase in Golgi bodies.

GOT in mitochondria.

Plasma enzyme level depends on:

(A) The rate of enzyme release form the cell. This depends on cell damage, cell proliferation and the degree of enzyme induction by inducers as phenobarbitone.

(B) The rate of enzyme clearance from the plasma.

* Small enzymes are removed by the kidney as α-amylase.
* Large enzymes are removed by proteases.

Localizationof tissue damage by enzymes:

= Tissue of origin of non- plasma specific enzymes.

- The increase in the level of certain enzymes in the plasma helps in localization of the diseased or damaged tissue or organ " It helps in diagnosis ".

-Most of the clinically important enzymes are common to all cells, yet. some enzymes are located at high concentration in certain tissue,e.g.

Creatine kinase is located predominantly in muscles.

γ-GT is located predominantly in liver

GOT is located predominantly in heart.

GPT is located predominantly in liver.

-Localization of cell damage is improved by:-

(A) Isoenzyme determination.

Iosenzymes (isozymes) of an enzyme differs in their concentrations in the different tissues. Their increase in blood helps in exact localization of the affected tissue.

Examples:

LDH-1 arises mainly from the heart

LDH-5 arises mainly from the liver.

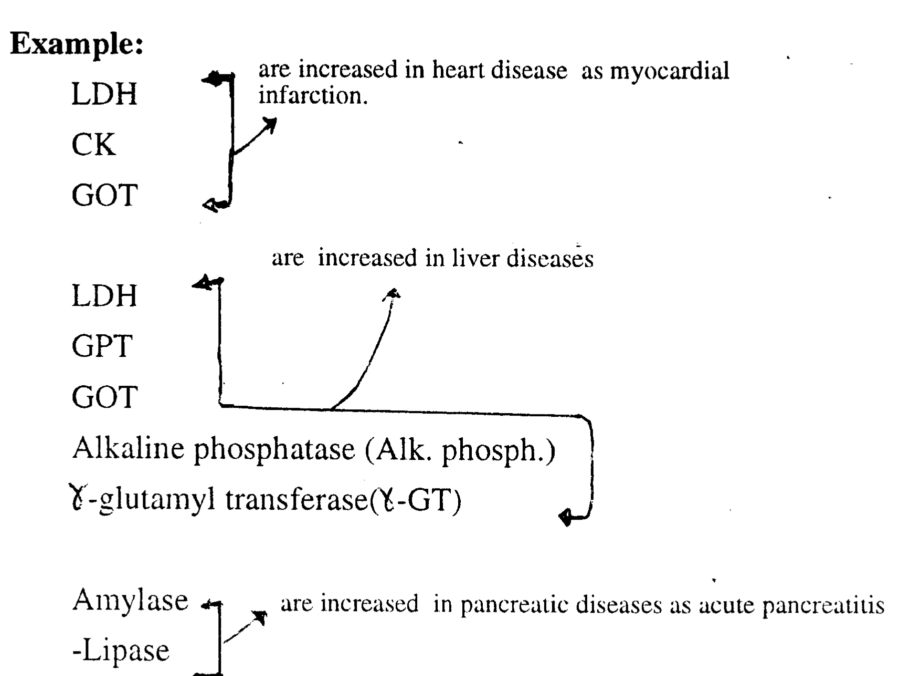
CK-MM arises mainly from the skeletal muscles.

CK-MB arises mainly from the heart.

CK-BB arises mainly from the brain.

(B)Estimation of more than one enzyme "Multiple enzyme determination" .

Example:



Causes of elevated plasma enzyme levels:

1. Pathological

* Cell damage by tumours or trauma

* Inflammation causes increased cell membrane permeability and release of enzymes from the cell to the blood.

2. Physiological causes :

-Alkaline phosphatase is moderately increased in pregnancy " from the placenta" and in growing children " from the growing bones.

-Transaminases and CK are increased in pregnancy "late" and in labour.

3. Induction of enzymes by Drugs:

Barbiturates " phenobarbitone" and phenytoin and cimetidine are inducers of many enzymes especially the microsomal enzymes as γ-glutamyl transferase (γ-GT).

4. Artifactual:

Hemolysis of RBC's into the plasma causes release of more enzymes from the RBC's into the plasma.

Units of enzyme activity :

The concentration of enzymes are measured as units of activity.

The unit is the quantity of enzyme that catalyse the conversion of one micromole (μmol) of substrate per minute. It is measured by the rate of the reaction.

It is expressed as unit /ml or litre(U/L or U/ml.)

VITAMINS

Vitamins are organic compounds which are essential for life. Vitamins were given this name by Casimir Funk in 1911. Funk had found that a substance present in the outer husk of rice was effective in curing and preventing the deficiency disease beriberi. Because this substance was essential "vital" for life and contained amino group, Funk gave it the name"vita/amine" which was later modified to the term vitamins.

Vitamins were referred to by letters and numbers denoting the sequence of their discovery.

Vitamins have the following characters:

1. They are present in the natural food as vitamins or in some types as precursors " provitamins".

2. They are required in small amounts .

3.They cannot be synthesized by the body or their synthesized quantities are not generally sufficient.

4. They must be supplied by the diet .

5. They don't enter in the structure of the cell.

6. They do not produce energy.

7. Some vitamins occur in more than one chemical form called vitamers as vitamin D 2 and D3".

Classification:

Vitamins are classified into two groups according to their solubility :

(A) Fat soluble vitamins. They include :

1. Vitamin A "Retinol or antinight blindness factor "

2. Vitamin D " Cholecalciferol or anti-rachitic vitamin "

3. Vitamin E " Tocopherol or anti-sterility vitamin in Rats"

4.Vitamin K "napthoquinones or antihemorrhagic" koagulation" vitamin.

(B) Water soluble vitamins. They include

a. Vitamin -C " L-ascorbic acid "

b. The B-complex of vitamins.

The members of the B-complex vitamins include.

1. Thiamin " Vitamin Bl".

2. Riboflavin " vitamin B2".

3. Niacin " nicotinic acid or vitamin B3"

4. Pantothenic acid " vitamin B5 "

5. Vitamin B6 "Pyridoxine".

6. Biotin " Cocarboxylase" vitamin H.

7. Folic acid " Folacin"

8. Vitamin Bl2" cyanocobalamin"

9. Para-Amino Benzoic Acid " PABA".

Fat Soluble Vitamins
Vitamin A

The active forms of vitamin A are :-

1. Retinol

   Vitamin A alcohol

2. Retinal

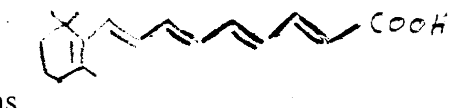
   Vitmain A aldehyde

   also called Retenine

3. Retinoic acid

   which is important for

   synthesis of glycoproteins.



The provitamin A in vegetables is called carotene which is yellow pigment present in many vegetables as carrot, apricot, tomatoes.

Sources:

1. Liver, egg yolk , butter, whole milk are good sources of preformed Retinol. Retinol is mainly present as ester with palmitic acid.

2. Yellow and dark green vegetables as carrots, apricots, tomatoes , green beafy vegetables,....

These vegetables contain the provitamin carotene " as ß-carotene".

* Requirements: Male 5000 I.U. (1000 retinol equivalents RE).

Female 4000 I.U. (800 RE).

N.B.: One RE = 1 µg retinol and 6 µg ß-Carotene.

Absorption:

*The preformed retinol is absorbed with the fats.

* ß-Carotene is oxidized in the intestinal cells to form 2 Retinal molecules .

-Also , in the intestinal mucosa Retinal is reduced to Retinol by a NAD(P) specific reductase.

-Small amounts of Retinal are oxidized to Retinoic acid.

-ß-carotene can also be directly absorbed.

Storage :

Vitamin A "Retinol" is stored in the liver. It is released into the blood attached to binding protein.

Liver stores of vitamin A can be sufficient for several months.

ß-carotene

ß-carotene Dioxygenase

2 X

C=O H

Retinal (Retinaldehyde)

Reductase

NAD(P)H+H+

NAD(P)

Oxidase

Retinal

Retenoic acid

Function of Vitamin A

Retinal , Retinol, Retinoic and the provitamin ß-carotene all have Unique biological function.

Retinal	Retinol	Retinoic	ß-Carotene
*It is a component of the visual pigment Rhodopsin which is important for Vision in dim light.	*It acts as a steroid hormone. * It maintaines reproduction in males and females. * It maintains epithelial tissue and mucus secretion.	*It participates in glycoprotein synthesis. * It supports growth and differentiation of tissues.	*It is an antioxidant esp. at low O2 tension. * It may have anticancer effect

Role of Vitamin A in Vision

* Retinal "retinaldehyde" is a component of the visual pigment Rhodopsin.

*Rhodopsin is present in the rod cells of the retina. These cells are responsible for vision in poor (dim) light.

*Rhodopsin is formed of the protein Opsin linked to ll-cis Retinal.

* When rhodopsin is exposed to light the following occurs.

1. The ll-Cis Retinal is changed to all-trans-retinal isomer. This isomerization produces changes in the conformation of retinal.
2. Rhodopsin dissociates to opsin and all-trans-retinal.

*These conformational changes in Rhodopsin caused by light "photons" cause rpid influx of calcium ions which initiates a nerve impulse through the nerve ending in the retina (i.e. light perception by the brain).

* Vision in Dim light depends on regeneration of Rhodopsin. The all-trans-retinal is isomerized to ll-cis-Retinal that binds to opsin to form Rhodopsin.

Rhodopsin ((11-cis retinal+opsin))

Bright light

opsin

Dim light "Dark"

All-Trans- Retinal

Isomerase

$NAD(P)H+H^+$

Retinal reductase

NAD(P)

11- Cis - Retinal

Retinol

in equilibrium with blood retinol

Role of Vitamin A aldehyde " Retinal" in vision.

Deficiency manifestation

1. Night blindness (nyctalopia)

Inability to see in dim light occurs due to inability of the rtina to form Rhodopsin in the rod cells. Rhodopsin is formed of opsin + 11-Cis retinal.

2. Xerophthalmia = dryness of the eye due to keratinization of the epithelium of the lacrimal glands.

N.B.: Xero = dry

3. Keratomalacia = keratinization of the cornea complete loss of vision may occur.

4. Xerodermia = dry, rough and scaly skin.

4. Dryness and death of the epithelium of the respiratory, gastro-intestinal and genitourinary system.

This causes repeated infections in these systems.

Toxicity of vitamin A= Hypervitaminosis A

*This occurs as a result of over dosage (large amounts of retinal (50,000 IU) for long period).

* Signs of toxicity include enlarged liver, severe headache, peeling of skin, bone and joint pains.

Vitamin D "Cholecalciferol"

Chemistry of Vitamin D

* Vitamin D is a steroid derivative. It is a thermostable fat soluble vitamin.

* There are two forms of Vitamin D " D_2 & D_3" Vitamin D_2 is formed in plants and vitamin D_3 is formed in animals.

Sources:

1. Fish liver oils, eggs, liver
2. Milk is a poor source of vitamin D.
3. The provitamins ergosterol in plants and 7-dehydrocholesterol in animals are changed to the vitamin D_2 and D_3 respectively by exposure to sunlight.

Sun light

7-dehydrocholesterol "under the skin" → Vitamin D_3 "Cholecalciferol"

Sun light

Ergosterol in plants → Vit. D_2 ergocalciferol

Function and mechanism of action:

The active form of vitamin D_3 "1,25 dihydroxychol-calciferol" produces the following effects:

1. It stimulates calcium absorption from the intestine.

2. It stimulates calcium reabsorption from the renal tubules, thus, decreasing calcium loss in the urine.

3. It helps deposition of calcium in the matrix of the bones and teeth.

4.In cases of severe hypocalcaemia especially when the dietary calcium is low, Vitamin D stimulates calcium resorption from bones into the blood to normalize the level of the blood calcium.

Mechanism of action:

Both forms "D_2 and D_3" are effective and are metabolised identically by the body. Vitamin D_3 whether exogenous or endogenously formed , is carried in the blood bound to a plasma binding α-globulin called D-binding protein (DBP). In the liver microsomes, 25-hydroxylase enzyme adds-OH group to the 25 carbon of vitamin D_3 to form
25-hydroxycholcalciferol. this form of the vitamin represents the circulating form in the blood.

If the blood calcium level decreases below the normal, the parathyroid hormone " parathormone is increased to stimulate a 1-hydroxylase enzyme in the mitochondria of the renal tubular cells which adds another OH group at position 1 in the 25-hydroxy-vitamin D_3 . this active form is also called calcitriol. It acts like a steroid hormone . The active form enters the target cell and bind to an intracellular protein receptor. The vitamin

receptor complex stimulates the synthesis of the calcium binding protein "calmodulin" which enhances calcium absorption in the intestine and renal tubules.

Deficiency diseases of vitamin D

(A) In children: The disease is Rickets.

The causes of rickets could be :

1. Deficiency of vitamin D_3 in food
2. Little exposure to sunlight when the child is kept in doors.

The manifestations of rickets include:

1. Boxy skull.
2. delayed teething
3. Pigeon chest.
4. contracted pelvis
5. Bowing of the legs.
6. delayed standing and delayed walking.

(B) In adults: the disease is osteomalacia.

The bones become fragile and brittle.

Fractures of bones occur very easy on minor trauma.

Recommended Daily Allowances:

5 μg " 200 IU" for adults.

10 μg " 400 IU" for children and lactating females.

Hypervitaminosis D

High dose of vitamin D as 4000 IU/day for long time causes toxicity. The early signs of toxicity include nausea, vomiting diarrhoea, headache. Prolonged hypervitaminosis leads to calcification of soft tissues as the kidney, arteries and the liver leading to renal damage, hypertension and liver damage.

VITAMIN E (Tocopherols)

(Rat antisterility factor)

Chemistry:

Vit. E includes 8 naturally occurring tocopherols .

The D-α Tocopherol is the most active form.

*Tocopherols are derived from tocol nucleus.

* Tocopherols are strong antioxidants because they can be readily oxidized to prevent oxidation of other materials "as fatty acids in fats."

Sources:

* Green plants

* Plant oils

* Eggs and liver

Vitamin E is destroyed by commercial cooking and food processing including deep-freezing. It is better to get it from fresh sources.

Function:

1. Antioxidant function:

* It is readily oxidized in the presence of oxygen, therefore it prevents oxidation of polyunsaturated fatty acids in the cell membrane.

2. It is antisterility factor in rats.

3. It prevents hemolysis of RBC's, necrosis of liver cells and atrophy of muscles.

Deficiency manifestation:

*Vit. E deficiency can be reflected on the antioxidant function that lead to hemolysis of RBC's , necrosis of liver cells and atrophy of muscles.

* Vitamin E, selenium, glutathione together withvit. C and A are important in removal of the free radicles from the cells.

* Vit. E deficiency in rats can cause sterility which is not prooved in humans.

* **Recommended Daily Requirements :**

8-10 mg (15 IU)

* **Hypervitaminosis**

Vitamin E is the least toxic of all fats soluble vitamins.

Vitamin K

Chemistry : There are 3 types of vitamin K. All types are derived from naphthoquinone

1. Vitamin K_1.

* It is fat soluble

* It is present in plants esp. green leafy plants as spinach, alfa-alfa, cabbage, lettuce.

2. Vitamin K_2

*It is fat soluble.

* It is formed in the intestine by intestinal bacteria.

3. Vitamin K_3 = menadione

* It is **water** soluble

* It is synthetic vitamin

Function :

1. Vitamin K is required for the formation of γ- carboxyglutamate in prothrombin, factor VII, factor IX and factor X.

Vitamin K is an important coenzyme necessary for γ -carboxylation of glutamic acid in the clotting proteins (II,VII,IX acid X). The γ-carboxyglutamate can bind effeciently Ca^{++} . This binding helps in release of platelet factor that is necessary for blood clotting .

Dicumarin and warfarin can inhibit blood clotting because they inhibit the γ-carboxylation of glutamic acid in these clotting proteins by inhibiting the regeneration of the active form of Vit. K.

2. Coenzyme Q has structural similarity to vitamin K. CoQ is essential component of the respiratory chain of mitochondria.

How can deficiency of vitamin K occur?

1. In Newly born infants.

* Infants are born with sterile intestine

* It is important not to operate on newly born infants because they are naturally defecient in Vitamin K, especially in the first week of life.

2. Long treatment with intestinal antiseptics (as Neomycin and streptomycin) Kill the intestinal bacteria (bact. flora) that form Vit. K_2.

3. Liver diseases:

Vitamin K act in the liver to form the mature clotting factors by making γ-carboxylation of glutamic acid.

4. Malabsorption of fats and fat soluble vitamins as in deficiency of bile salts (obstructive faundice) or in steatarrhoea.

5. Prolonged treatment with anti-vitamin K as Dicumarols and warfarin.

Menadione "Vit.. K3" is an antidote of Diumarol overdosage.

Effect of Vitamin K deficiency:

Its deficiency causes haemorrhages due to defective blood clotting.

Daily Requirments:

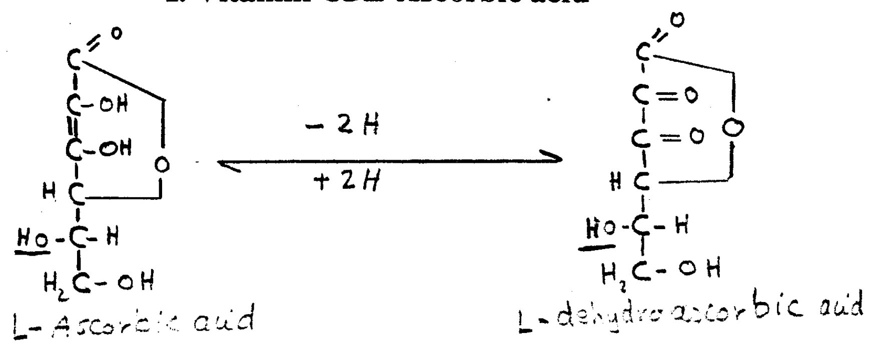
70-140 μg/day

Toxicity = hypervitaminosis of Vit. K.

Large doses for long period can cause jaundice.

Water soluble vitamins

I. Vitamin C=L-Ascorbic acid



*Vit. C is a derivative of glucose that changes first to L-gulonic acid. In animals "Except man, quinea pig, indian pat and some birds" L-gulonic acid can change to L-ascorbic acid .

* It is water soluble . It is affected by heat " heat labile"

***Sources:** Rich sources of vitamin C are :

1. Guava , citreous fruits as lemon, orange, mandarine, grape fruit.

2. Green pepper, mango. Vitamin -C should be ingested fresh.

* **Importance:**

Vitamin -C is a reducing agent . It is important for the function of the enzyme that contain iron in the ferrous state as:

1. Proline and lysine hydroxylases.

2. Cytochrome oxidases.

3. Hydroxylases used in adrenaline synthesis "dopa hydroxylase ".

4. 7 α-hydroxylase of cholesterol.

Vitamin -C keeps the iron of these enzyme in its active ferrous states.

Important functions of Vit. C includes

1. It is important for iron absorption in the intestine. It changes the ferric iron of the food into ferrous form that can be absorbed.

2. It is essential for the normal synthesis of mature collagen. Mature strong collagen is formed by certain modifications that occurs in the collagen after its synthesis in the ribosomes. One of these changes is the formation of hydroxyproline and hydroxylysine.

$$\text{Lysine} \xrightarrow[\text{Vitamin -C}]{\text{Lysine-hyroxylase}} \text{hydroxy lysine}$$

$$\text{Proline} \xrightarrow[\text{Vit. C}]{\text{Proline-hydroxylase}} \text{hydroxyproline}$$

The strong normal collagen can protect and support the blood vessels and nerve ending and the other components of the connective tissue.

3. Vitamin -C is important for the synthesis of
   (a) Noradrenaline and adrenaline
   (b) Corticosteroid hormones
   (c) Bile salts from cholesterol
4. Vitamin-C raises the immunity against diseases. Vitamin C may be necessary for the synthesis of the complements which are similar to collagen in structure.
5. Vitamin-C is considered as anti-oxidants because it helps in removal of the free radicals.

Deficiency manifestation:

* Deficiency causes Scurvey. The deficiency occurs after 2-3 weeks of lack of the vitamin in food.

The manifestation of scurvey are all due to deficient formation of the normal strong collagen, they include .

1. Petichae--> minor spots of haemorrhage under the skin due to increased fragility of capillaries under the skin on minor trauma .

2. Swollen and bleeding gums on brushing the teeth.

* Iron deficiency anaemia due to defective iron absorption.

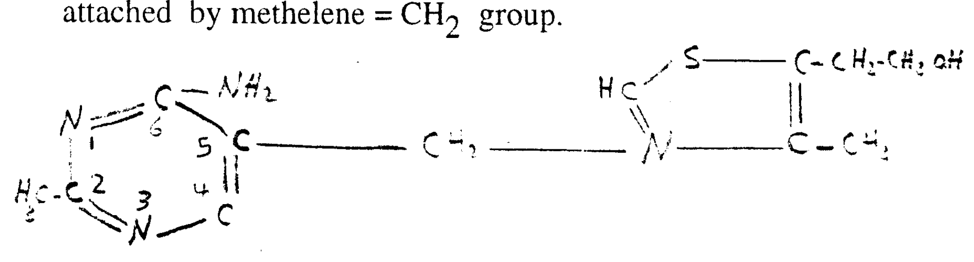
II.THE B-COMPLEX OF VITAMINS

The vitamins of the B-complex include the following types:

I- Thiamin = Vitamin B_1 = Anti beri-beri

Properties:

*It consists of substituted pyrimide ring + thiazol ring attached by methelene = CH_2 group.



(2methyl-6 amino pyrimidine) Thiazol

* Stable at 100°C and in acid solution.

Sources:

1. In yeast " high concentration"

2. liver, kidney, spleen.

3. Vegetables and fruits

4. Unrefined cereal grains (in the bran)

Functions:

It forms thiamine pyrophosphate "TPP" with phosphoric acid in the tissues T.P.P. is a coenzyme in :

1. decarboxylation reaction " oxidative"

2. transketalose reaction.

These two reactions are important in carbohydrate metabolism. Deficiency leads to Beri Beri

2 types of beri beri are known.

1. Wet beri beri is manifested by :

* Congestive heart failure.

* Oedema.

* Peripheral neuritis.

2. Dry berib beri -----> atrophy of the peripheral nerves (Peripheral neuritis).

Requirements:

1.5 mg/day for adult

2 mg/day for pregnant women.

Vitamin B_2 (Riboflavin)

Properties: It is formed of

1. Flavine pigment.

2. D-ribitol (formed by reduction of ribose)

Flavine

Ribitol

It is heat stable yellowish compound. It is very sensitive to light.

Sources:

1. Milk, 2. egg, liver, spleen, kidney , 3. yeast.

Functions:

It gives F.M.N. = flavin mononucleolike

F.A.D.= Flavine-adenine dinucleotide

They functions as hydrogen carriers

They are coenzymes for flavoprotein enzymes

I. F.M.N. is a coenzyme for

L-amino acid dehydrogenase

II. F.A.D. is a coenzyme for

* D-amino acid dehydrogenase .
* Glycine oxidase.
* Xanthine oxidase
* Acyl COA dehydrogenase.

Deficiency :

Deficiency of riboflavin leads to :

1. Cheilosis (dry lips)
2. Angular stomatitis.

3. Seborrhoeic dermatitis of face.

4. Vascularisation of the cornea.

Requirement:

1.7 mg/day for adult

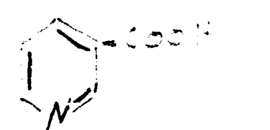
Pregnant woman : 2 mg/day.

3. Nicotinic acid "Vitamin B3"

" Niacin" - A pellagra preventive factor

Properties :

* It occurs as amide form in the tissues "nicotinamide"



Nicotinic acid

Nicotinamide

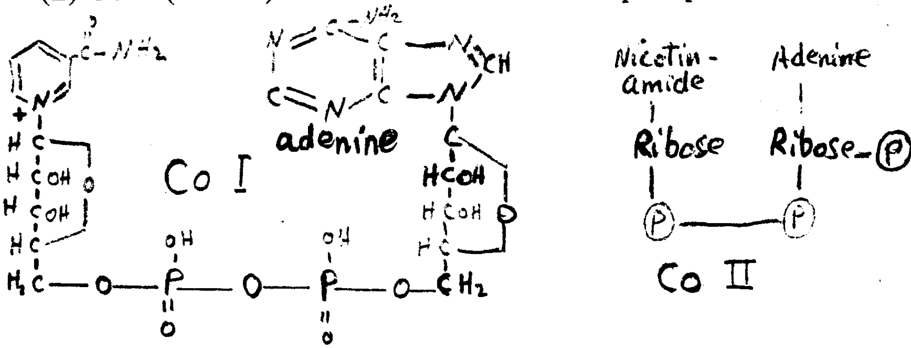
Function:

It enters in the formation of a group of coenzymes which act as hydrogen carriers.

These are:

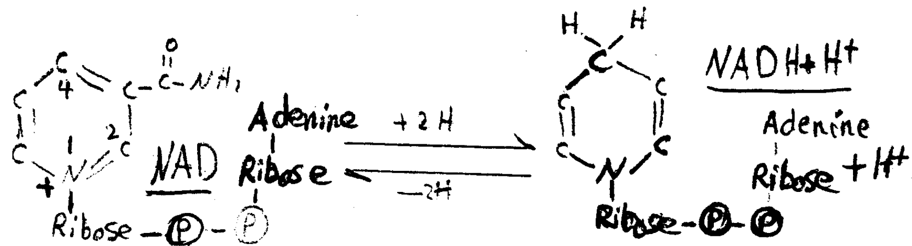
(1) Co I (NAD) = Nicotinamicde acdenine dinucleotide

(2) Co II (NADP) = Nicotinamide adenine phosphate.



3. Co III = Nicotinamide- Ribose- (P)

Co I & II & III act as Hydrogen carriers for the dehydrogenases.



Deficiency: Deficiency of nicotinic acid leads to pellagra "rough skin"

It is manifested by

1. Dermatitis : rough, dry, scaly skin .
2. Diarrhoea
3. Dementia: Loss of mental power

Requirements:

Adult 15 mg/day

Pregnant female : 17 mg/day

Sources:

1. Yeast liver, kidney, pleen

Vegetables, Milk, eggs.

2. Tryptophan amino acids. It is changed in the body toNiacin.

* Maize is poor is Niacin and tryptophan.

4. Folic acid " Folacin"

Properties :

It consists of

1. Petridine nucleus
2. Para-amino-benzoic acid (PABA)
3. Glutamic acid " one or more residues"

Function: The active form of folic acid is tetrahydrofolate the folate is reduced mainly in the intestine into dihydrofolate and then to tetrahydrofolate (THF) .

Folic acid --reductions (NADPH+H$^+$)--> dihydrofolic acid --Folate reductase (NADPH+H$^+$ → NADP)-->(THF)

THFacts as carrier for one carbon fragments as methyl "CH3" , methylene (CH_2) and Formyl (-C(=O)-H). The one carbon fragments are used for synthesis of important compounds as methionine from homocysteine and thymidylate "TMP" from d-UMP. TMP is needed for DNA synthesis and maturation of erythrocytes.

Deficiency : can occur in the following conditions:

1. Haemolytic anaemia.
2. During pregnancy and lactation due to increased demands.
3. Treatment of malignancy by dihydrofolate reductase inhibitors as methotrexate.

Anaemia due to folic acid deficiency is megaloblastic anaemia (macrocytic anaemia) due to defects in erythropoesis.

Sources:

Green leafy vegetable, oranges, whole grain cereals, liver, kidney and yeast. The name folacin is derived from the latin word for leaf"foliage"

Requirments:

Adult 0.5 mg/day.

Pregnant 0.7 mg/day

5. Pantothenic acid " Vit. B5"

Properties :

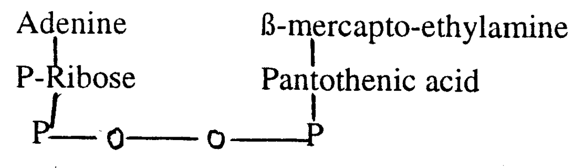
It is formed of combination of pantoic acid and ß-alanine .

$CH_2(OH)—C(CH_3)_2—CH(OH)—C(=O)\,\vdots\,—NH—{}^{\beta}CH_2—{}^{\alpha}CH_2—COOH$

pantoic acid ⋮ ß- alanine .

Function:

It is a component of coenzyme A and acyl carrier protein



COA is an acid carrier. COA is important in activation of fatty acids for ß-oxidation. Acetyl -COA is used in kreb's

cycle,cholesterole synthesis, fatty acid synthesis and acetylcholine synthesis and others.

Deficiency leads to

1. Anaemia (Normocytic anaemia)
2. GIT disturbances, insomnia, burning cramps and headache.

Sources -Yeast , milk , meat, liver,

Daily Requirement: 10 mg/day

6. Vitamin B_6

Properties:

Vitamin B_6 consists of 3 pyridine derivaties:

Pyridoxine, pyridoxal and pyridoxamine

Pyridine Pyridoxine Pyridoxal Pyridoxamine

Pyridoxal phosphate is the active coenzyme form of that vitamin.

Pyridoxal --Kinase (ATP → ADP)--> pyridoxal phosphate CH_2-O-P

Pyridoxal phosphate is given the abbreviation (PLP)

Sources:

Liver, yeast, whole grain cereals , fruits and vegetables.

Functions:

Pyridoxal phosphate is a coenzyme for the following reactions:

I. Transamination reactions.

Pyridoxal phosphate carries the amino group from the amino acid to form pyridoxamine phosphate which then transfers its amino group to the α-keto acid.

The common exqamples of transamination reactions are the reactions catalysed by the amino-transferases GOT and GPT.

Glutamic acid + pyruvic <---(GPT / PLP)-----> α-ketoglutaric + alanine .

Glutamic acid + oxalacetic <---(GOT / PLP)-----> α-ketoglutaric+ aspartic.

2. Decarboxylation reactions of amino acids.

Amino acid -----(decarboxylase / PLP CO_3)----> amines.

Examples of amino acid decarboxylases:

Histidine -----(decarboxylase / PLP CO_2)-----> Histamine

OH-Tryptophane -----(decarboxylase / PLP CO_2)-----> OH-tryptamine (Serotonin)

Glutamic -----(decarboxylase / PLP CO_2)-----γ- aminobuteric " GABA"

3. -AminoLevulinic acid synthesis by ALA -synthase is the first reaction in Heme synthesis that need pyridoxal phosphate. Deficiency of pyridoxine is therefore a cause of anaemia.

4. Glycogenolysis in the muscles by glycogen phosphorylase depends also on pyridoxal phosphate.

Deficiency of Vitamin B_6: Causes and manifestations

Deficiency of Vit. B6 due to lack in the food is very rare. Deficiency can be caused by prolonged use of the oral contraceptives, in alcoholism and in prolonged use of the antituberculous drug isoniazide which form a hydrazone with pyridoxal. The use of isoniazide without concomitant use of vitamin B6 may cause convulsions due to deficiency of GABA in the brain tissue. anaemia nausia niacin synthesis

7. Vitamin B_{12} "Cobalamin"

Vitamin Bl2 has a complex ring structure attached to Cobalt ion in the centre. The cobalt ion may be attached to cyanide to form cyanocobalamin or OH group to form hydroxycobalamin or 5-deoxyadenosine to form 5-deoxyadenosylcobalamin or methyl group to form methyl- cobalamin.

Sources:

1. The vitamin is synthesized by microorganisms as the intestinal bacterial flora of animals and is stored in their livers. Liver is therefore a good source of vitamin Bl2 . The vitamin exist in animal's liver as methylcobalamin and adenosylcobalamin.

2. The vitamin is absent from plants.

3.The commercial preparation of the vitamin is cyanocobalamin.

Intestinal Absorption of Vitamin B_{12}:

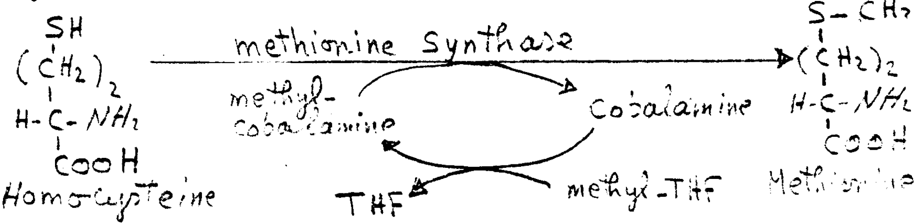
The absorption of vitaminB_{12} occur in the distal ileum in the presence of a specific glycoprotein called the intrinsic factor

which is secreted by the parietal cells of the gastric mucosa. After absorption into the blood, the vitamin is bound to a plasma protein called transcobalamin II. In the liver the vitamin is stored bound to transcobalamin I.

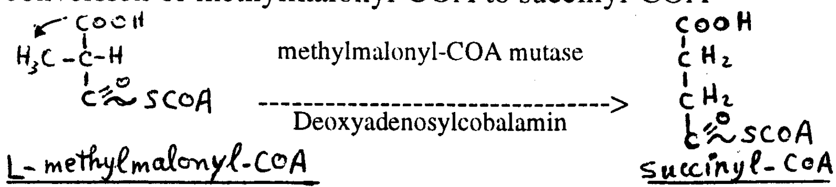
Functions of Vit. B_{12}

The active B12 coenzymes are the methyl cobalamin and the deoxy adenosylcobalamin. Vitamin B12 coenzymes are important for 2 essential biochemical reactions, namely,

1. Methylcobalamin is the coenzyme for the conversion of homocysteine to methionine. In the same reaction methyl tetrahydrofolate (CH_3-THF) changed to tetrahydrofolate (THF) the benifits of this reaction are that the stores of the essential amino acid methionine are maintained and the tetrahydrofolate form of folic acid is maid available for the synthesis of the purines and pyrimidine nucleotides that are then usedfor DNA synthesis and cell divison .



2. Deoxyadenosylcobalamin is the coenzyme for the conversion of methylmalonyl-COA to succinyl-COA



This reaction is important for oxidation of fatty acids with odd numbers of carbon atoms to form succinyl-COA that is further metabolised through the Kreb's cycle.

Deficiency of vitamin B_{12}

Vitamin B_{12} stores in the liver could be sufficient to supply the body needs for years before deficiency manifestations occurs even in complete absence of vitamin intake.

The causes of deficiency are:

1. Strict vegetarians "Vegans" do not eat any foods of animal origin as liver, egg , milk or meat. Plants are devoid of this vitamin.

2. Lack of the intrinsic factor as in gastrectomy or in the presence of intrinsic factor blocking antibodies or serum gastric parietal cell autoantibodies "autoimmune disease". All these cases prevent vit. B_{12} absorption leading to Pernicious anaemia.

3. Impaired vitamin B_{12} absorption by drugs as neomycin, para-aminosalicylic, anticonvulsants, phenformin and Alcohol.

*The disease caused by deficiency of Vit. B12 is megaloblastic anaemia accompanied by neurological manifestations caused by nerve degeneration.

The megaloblastic anaemia is caused by defect in the methionine synthase reaction. The methyl tetrahydrofolate is not changed into tetrahydrofolate leading to its deficiency. This is a consequence of folate being trapped as methyltetrahydrofolate ,"folate trap".

The deficiency of tetrahydrofolate causes decrease in purine and pyrimidine nucleotide synthesis and impaired DNA synthesis and cell division with accumulation of megaloblasts in the bone marrow. The peripheral blood picture shows macrocytic anaemia and hypersegmented neutrophils . The serum B12 level is less than 100 pg/ml. The neurological manifestation are mainly due to

lack of the role of B_{12} in lipid metabolism, thus, affecting the myelin sheath of the nerves. The term pernicious anaemia is given to the deficiency of vit. B_{12} due to lack of intrinsic factor. This disease is a hereditary autoimmune disorder mostly seen after the age of 35 in northern European ancestry and accompanied by atrophic gastritis.

Requirements:

Biotin – vitamin H

Biotin molecule contains imidazole and Thiophene rings Attached to Valeric acid.

Souxces :

1- Intestinal bacterial sysnthesis.
2- Natural foods.

```
            O
            ‖
            C
          /   \
    H - N       N - H
        |       |
    H - C ----- C - H
        |       |
      H2C       CH(CH2)4
          \   /   |
            S     COOH
```

Function : Biotin is a coenzyme of carboxylase Enzymes "co – carboxylase"

Biotion carry the co_2 and trensfer it to the substrates in the carbxylation reactions. E.g.

$$\text{Pyruvic} + co_2 + \text{ATP} \xrightarrow[\text{Biotin , Hg}^{++}]{\text{Pyruvate Carboxylase}} \text{oxaloacetic}$$

Biotin deficiency : defiaeny of biotin in food is very rare. Deficiency can be caused by consumption of large amounts of raw egg white which contains a heat lobile protein called Avidin. Avidin binds tightly with biotin perventing its absorption.

The manifestations of biotin deficiency are hallucination , deperession, dermatitis and musile pian.

Failure of binding of biotin to the carboxylases due to hereditary deficiency of the holocarboxylase synthase causes multiple carboxylase deficiency and biotin deficiency symptoms, Immune defiaency diseases may occur

NITROGEN BASES

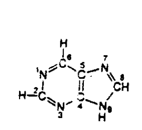
2 types of nitrogen bases are included in the structure of the nucleotides in the nucleic acids :

1. Purines 2 Pyrimidines

1. Purines

2 amin types "Adenine and Guanine" and 2 intermediates "hypoxanthine and xanthine" are the main purines. Uric acid is the end product of all the purines in man.

The purines are derived from the purine nucleus



Purine nucleus

Purine nucleus

The purine bases include:

(a) Adenine

6- Amino purine

(b) Guanine

2,amino,6 oxypurine

(c) Hypoxanthine

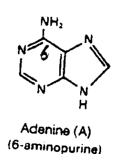
6-oxypurine

(d) Xanthine

2,6-dioxypurine

(e) Uric acid " end product"

2,6,8 trioxypurine



Adenine (A)
(6-aminopurine)

Guanine (G)
(2-amino-6-oxypurine)

Hypoxanthine
(6-oxypurine)

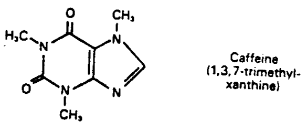
Xanthine
(2,6-dioxypurine)

uric acid

Unusual purine bases " minor bases"

N-6 methyl adenine
N-7 methyl guanine
] They occur in very small number in the nucleic acid of man and bacteric to protect it from lysis.

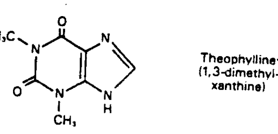
-Methylated xanthines in plants:



Caffeine (1,3,7-trimethylxanthine)

* Caffeine " Trimethyl xanthine"

" 1,3,7 trimethyl " "



Theophylline (1,3-dimethylxanthine)

* Theophylline " 1,3 dimethyl xanthine " in tea

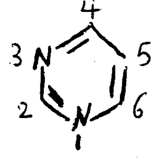
* Theobromine " 3,7 dimethyl xanthine " in cocoa

Theobromine (3,7-dimethylxanthine)

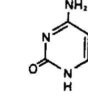
(2) <u>Pyrimidines</u>

3 main types are derived from the pyrimidine nucleus "ring".

They are uracil, thymine, cytosine .

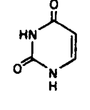


Pyrimidine nucleus



Cystosine (C) (2-oxy-4-aminopyrimidine)

Thymine (T) (2,4-dioxy-5-methylpyrimidine)



Uracil (U) (2,4-dioxypyrimidine)

(1) Uracil

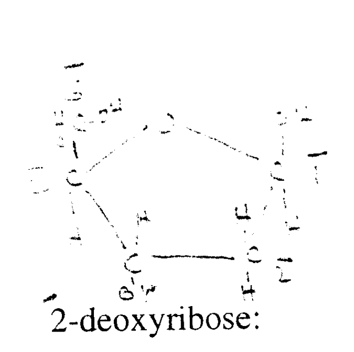
2,4-dioxy pyrimidine.

(2) Thymine = 5-methyl uracil

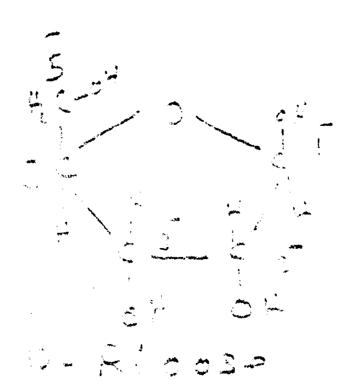
3. Cytosine:

2-oxy, 4-amino pyrimidine

Riboses in the nucleotide structure :



2-deoxyribose:



N.B.: The numbers of the atoms of the sugars in the nucleotides are primed by (′)

Phosphates in the nucleotide structure : H_3PO_4 " phosphoric acid " --> molecular formula

HO - P(=O)(OH) - OH ----> structural formula.

Nucleoside:

It is a compound formed of a nitrogen base and a pentose sugar " Ribose or 2-deoxyribose"

In purine nucleoside the bond is between N-number 9 of the nitrogen base and the C number 1' in the sugar.

In pyrimidine the bond is between N number 1 and the C number 1' in the sugar.

The bond between the nitrogen and the sugar is called N-glycosidic bond.

Examples of **nucleosides**

e.g. adenine nucleoside = Adenosine

Adenine - Ribose

Adenosine

e.g. uracil nucleoside = Uridine

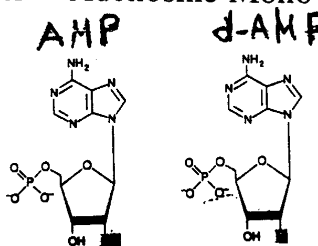
Uracil - Ribose

Uridine

Nucleotide structure

A nucleotide is formed of a nitrogen base attached to ribose or 2-deoxyribose which is attached to phosphate group at C-5 of the sugar.

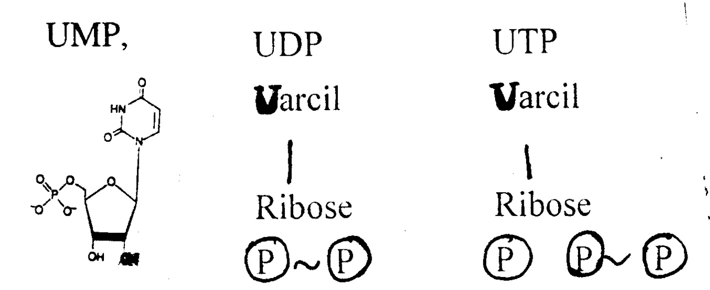
Example: AMP = Adenosine Mono Phosphate



ADP Adenine – ribose – Ⓟ~Ⓟ

ATP Adnione – rebose – Ⓟ~Ⓟ~Ⓟ

Pyrimidine nucleotides: examples



N.B. UTP = uridine Tri Phosphate

<u>The principle bases, nucleosides and nucleotides</u>

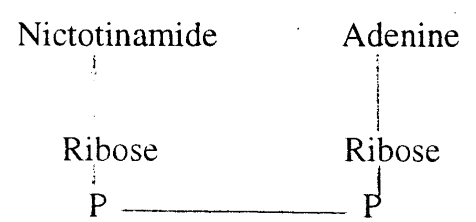
Nitrogen base	Nucleoside	Nucleotide
<u>Purines</u>		AMP "Adenosine Mono Phosphate"
Adenine (A)	Adenosine	or adenylic acid , ADP, ATP
Guanine (G)	Guanosine	GMP " Guanylic acid, GDP, GTP
Hypoxanthine	Inosine	IMP "Inosinic acid",
Xanthine	Xanthosine	XMP "Xanthinylic acid"
<u>Pyrimidines</u>		
uracil	Uridine	UMP = Uridylic acid. UDP, UTP
Thymine	Thymidine	TMP = Thymidylic " TDP, TTP
Cytosine	Cytidine	CMP = Cytidylic acid CDP, CTP

Importance of nucleotides

1. They enter in the structure of nucleic acid

2. They form the chemical energy stores in the cell as ATP and UTP to supply energy for certain chemical reaction in the cell. The energy released from hydrolysis of each of the 2 terminal phosphates of ATP is 7.1 Kcal/mole.

3. They enter in the structure of some coenzymes as NAD =Nicotinamide Adenine Dinucleotide



NADP = Niotinamide Adenine Dinucleotide Phosphate

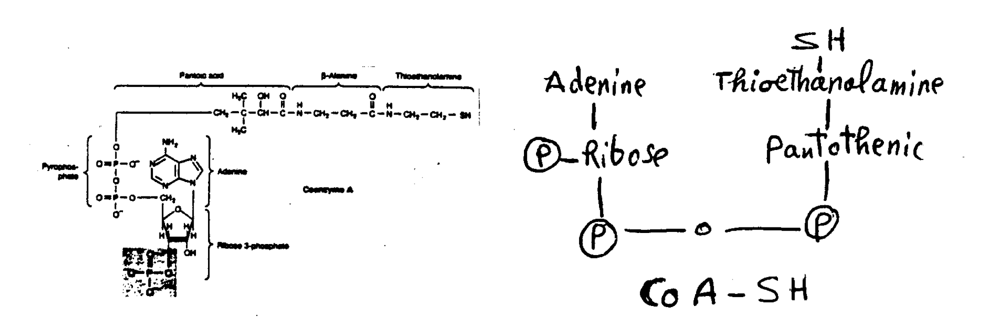
FAD = Flavine Adenine Dinucleotide

Flavine Adenine
Ribitol Ribose
P —————— P

FMN = Flavine mono nucleotide

Flavine - Ribitol - P

<u>COA-SH</u>



4. Some nucleotide act as mediators of hormonal action as cylic-AMP. C-AMP is synthesized from ATP by the action of adenylate cyclase enzyme.

$$ATP \xrightarrow{\text{Adenylate cyclase}} C\text{-}AMP$$

3',5'-Cyclic AMP

C-AMP produces a metabolic effects and has to be broken down rapidly by the enzyme phosphodiesterase

$$3',5'\ C\text{-}AMP \xrightarrow{\text{Phosphodiesterase}} 5'\text{-}AMP$$

5. S-adenosyl methionine is an important donor of methyl groups in transmethylation reaction.

methionine   Adenosine

Polynucleotide chain :

The polynucleotide chains form the structure of DNA "2 chains" and RNA"single chain". The single nucleotides are linked together within the chain by phosphodiester bonds. The

phosphate of one nucleotide is linked by ester bond to the OH group of C-3 of pentose of the previous nucleotide

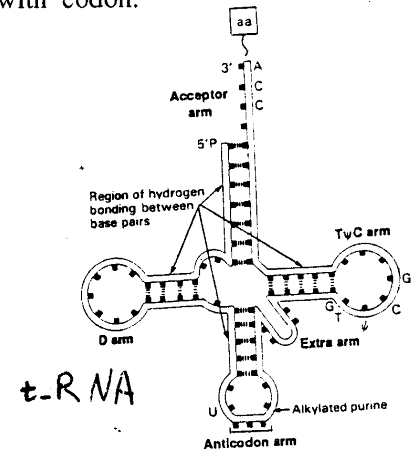
Differences between DNA and RNA:

DNA = Deoxyribonucleic acid.

it contains deoxyribonucleotides as d-GMP and d-TMP.

RNA = Ribonucleic acid. It contains ribonucleotides.

DNA	RNA
Location: DNA is present within the nucleus. The mitochondria contain few specific DNA.	**Location:** RNA is formed in the nucleus on a template of DNA and act in the cytoplasm.
Function: It stores the genetic information to be expressed as proteins. It also propagates the genetic information from the mother cell to the daughter cells.	**Fucntions** : The three types of RNAs have roles in protein synthesis . -The mRNA transmits the informations for protein synthesis from the nucleus to the ribosomes to be assembled according to the sequence of the codons to form a protein. -The r-RNA occurs in the structure of ribosomes. t-RNA contains the codons. It Carries amino acide to the ribosomes
Structure "Watson-Crik" model: DNA molecule is formed of 2 strands of polydeoxyribonucleotides. The two strands are coiled together to form a right handed double helix.	**Structure** RNA is generally formed of one single strand of polyribonucleotides . In t-RNA the single strand is folded on itself at some arms to form a double segments.

DNA	RNA
the nitrogen bases in the two strands are directed inwards facing each other and are held together by hydrogen bonds. According to the base-pairing rule the A in one strand is facing T in the other, while G should face the C. A === T G ≡≡≡ C	in which the bases are arranged according to the base-pairing rule A ======= U G≡≡≡≡≡≡≡ C
A is linked to T by two hydrogen bonds and G to C by 3 hydrogen bonds. The two strands of a DNA molecule are anti-parallel.  The two anti – parallel strands of DNA	The mRNA carries the codons as a sequence of 3 nitrogen bases. Anticodon sequence on the t-RNA is complementary with codon. 
Pentose sugar DNA contains the sugar 2-deoxyribose	**Pentose sugar** RNA contains the sugar ribose
Nitrogen bases: Adenine, Guanine, cytosine and thymine	**Nitrogen bases** Adenine, Guanine, cytosine and uracil.

October 6 University
Faculty of Dentistry

جامعة ٦ أكتوبر
كلية طب الأسنان

NOTES ON PRACTICAL BIOCHEMISTRY

By

PROF. ISMAIL HEGAZY

EXPERIEMENTS ON CARBOHYDRATES

I. Molisch's test :

It is a general test for all carbohydrates including monosaccharides, disaccharides and polysaccharides.

Produce of the test :

I. Put 5 ml of the carbohydrate solution in a clean test tube.

2. Add 2 drops of alcoholic α-naphthol and mix well.

3. Add carefully conc. sulphuric acid down the side of the test tube.

4. Appearance of the reddish violet ring at the junction of the two liquids indicates the presence of carbohydrates.On shaking, the whole solution turns violet in colour.

2. Iodine test :

This test is specific for the polysaccharides (Starch, dextrins).

Steps and observation:

I. Put 5 ml of the polysaccharide solution in a clean test tube.

2. Add 5drops of dilute iodine solution and mix.

3. Observation of

(a) Dark blue colour in case of starch.

(b) Reddish violet colour in case of dextrins.

(c) Gum arabic or any other carbohydrates give a negative result (the colour remains yellow).

3. Benedicts test :

This test is positive for all the reducing sugars as all the monosaccharides, lactose and maltose. Sucrose is non reducing sugar.

Steps and observation:

I. Put 5 ml of the Benedict's reagent in a clean test tube.

2. Add 8 drops of the tested solution and mix well.

3. Biol for 2 minutes and then cool.

4. The appearance of green, yellow, orange or red precipitate indicates the presence of a reducing sugar.

4. Barfoed's test:

This test differentiates between disaccharides (maltose, lactose, sucrose) and monosaccharides.

Steps and observation:

I. Put 5 ml of Barfoed's reagent in each of two test tubes.

2. Add 8 drops of the tested solution to one tube and 8 drops of distilled water to the other tube.

3. Boil in a boiling water both for 5 minutes.

Remove the two tubes and examine against a dark background for the appearance of Reddish precipitate in the tube that contain the tested solution which indicates the presence of Monosacharides.

4. Disaccharide give a positive result after longer time of boiling (> I0 minutes).

5. Ketose test :

This test is specific for ketoses as fructose and is used to differentiate between glucose and fractose.

Steps and observation:

I. Put 5 ml of the tested sugar (fructose) in a clean test tube.

2. Add 5 ml of conc. HCl and boil.

3. The appearance of an orange colour indicates the presence of fructose.

N.B.: Sucrose gives a positive result after longer time of boiling.

Experiments on Proteins

I. Biuret's test :

This test is a general test for proteins.

Procedure :

I. Put 5 ml protein solution in a clean test tube.

2. Add 5 ml of 10% NaOH solution and mix well.

3. Add copper sulphate 1% drop by drop and shake after each addition.

4. Pink or reddish violet colour indicates the presence of protein.

2. Heat coagulation test :

Albumin and globulins are coagulable protein.

I. Fill 2/3 of a test tube with the protein solution and heat gently the upper part of the test tube . Heat is not transmitted to lower part.

2. The appearance of a white opacities in the upper part of the tube indicates the presence of a coagulable proteins " albumin or globulin". If no coagulation occurs , the protein could be caseiogen, gelatin or peptone.

3. Full suturation test :

This test differentiates between albumin and globulin.

The procedure :

I. To 5 ml of protein solution add solid ammonium sulphate and shake well until dissolution. Repeat the addition of ammonium sulphate

and the shaking until the solution is fully saturated. Albumin is precipitated by full saturation.

4. Half saturation test :

I. Prepare a saturated solution of ammonium sulphate in distilled water.

2. In a clear test tube , add 5 ml of protein solution and add equal volume (5 ml) of the saturated ammonium sulphate solution and shake. The mixtur is now half saturated.

   Globulin is precipitated in half saturated solution.

Experiements on Fats

I. Solubility :

Fats are not soluble in water. Fats are soluble in the non-polar solvents as ether, benzene, petroleum ether.

I. Test the solubility of cotton seed oil in water. The oil separate on the top.

2. Test the solubility of the oil in ether. The oil is dissolved.

2. Grease stain test :

This test is a general test for fats.

I. Dissolve oil in ether.

2. Put drop of this solution on a filter paper and leave it to dry. Ether evaporates leaving the fat as a transparent spot on the filter paper.

3. Emulsification of fats by soaps.

Soaps and bile salts are emulsifying agents for fats. They change the water insoluble fats into emulsion in water and fats can then be carried by water.

1. Add 5 ml oil to 5 ml water. The oil is not dissolved and is separated on the top.
2. Add 5 ml of 1% soap solution to the mixture of oil and water. The oil is solubilized in water and form emulsion.

General Scheme for Idetification of Simple unknown solution of carbohydrates or proteins

[A] Physical properties :

Colour :

Odour :

Aspect : Clear or Tubid

Reaction : Neutral. acidic or alkaline.

[B] Chemical tests :

- Biuret test
  - Violet colour (+ ve) → Heat Coagulation test
    - Coagulation (Albumin or Globulin) → Precipitation by ammonium sulphate
      - Full saturation (Albumin)
      - Half saturation (Globulin)
    - No coagulation (Gelatin, peptone or caseinogen)
  - No violet colour (- ve) → Molishes test
    - No violet ring (- ve)
    - violet ring (+ ve) → Iodine Test
      - No blue colour → Benedict's test
        - green, orange or red colour (Reducing sugar) → Barfoed Test
          - Red ppt. after 10 min. (disaccharides)
          - Red ppt. in 5 min. (Monosaccharides)
        - No change in Colour (Sucrose)
      - Blue colour (Starch)

Evaluation Sheet I

Name : .... Sudent's Number.

Sample Number: ....

Report on Unknown Solution

(A) Physical characteristics:

1. Colour ....
2. Odour ....
3. Aspect ....
4. Reaction ....

(B) Chemical tests:

Test	Observation	Results